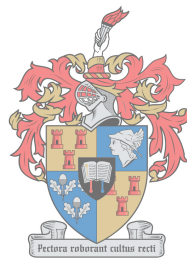


A study on the biological and physiological traits of *Bactrocera dorsalis*, with special reference to its invasion potential into the Western Cape of South Africa.

by

Welma Pieterse



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UNIVERSITY

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1918 · 2018

Dissertation presented for the degree of
Doctor of Philosophy (Agricultural Sciences)

at

Stellenbosch University

Department of Conservation Ecology and Entomology, Faculty of
AgriSciences

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March 2018

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Date: 26 February 2018

Summary

Bactrocera dorsalis (Hendel) is of Asian origin and is present in the northern and north-eastern parts of South Africa, but is still absent in other areas of the country including the Western Cape Province. The Western Cape Province is the largest producer of deciduous fruit in South Africa, exporting 41% of the deciduous fruit grown in the province. South Africa earned about R7 billion in export revenue from deciduous fruit exports in 2015. Currently, *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* s.l. Karsch are economically the most important fruit fly species on deciduous fruit in the Western Cape Province of South Africa. However, there is currently a lurking threat of potential introduction of *B. dorsalis* in Western Cape Province and this is of great concern to the deciduous fruit industry. *Bactrocera dorsalis* has shown remarkable range expansion over the past 10 years within Africa, adapting to different climatic conditions. *Bactrocera dorsalis* was also found to be able to out-compete a number of *Ceratitis* species in Africa. The aim of this study was to determine the invasive potential of *B. dorsalis* in the Western Cape Province of South Africa. The thermal biology, utilisation of deciduous fruit and the competitive ability of *B. dorsalis* were studied. A simple morphology based identification tool for *B. dorsalis* larvae was also developed in order to aid in early detection of the pest.

A detailed assessment of acute high and low temperature survival ability of four life stages of *B. dorsalis* and the plasticity thereof was carried out to test the hypothesis that traits of the thermal niche have contributed to the species' invasion ability. The extreme low and high temperatures at which c. 20% of the population of *B. dorsalis* survived were determined to be -6.5°C and 42.7°C, respectively, when using 2 h exposures. The egg stage was found to be the most resistant life stage to both high and low temperatures with $44 \pm 2.3\%$ and $60 \pm 4.2\%$ surviving the low and high discriminating temperature treatments respectively. The potential for adult hardening responses to mediate tolerance of extremes was also considered using a diverse range of acute conditions (using 2 h exposures to 15°C, 10°C and 5°C and 30°C, 35°C, 37°C and 39°C as hardening temperatures, and some treatments with and without recovery periods between hardening and discriminating temperature treatment). The results of these studies showed that although some significant hardening responses could be detected in certain treatments (e.g. after exposure to 37°C and 39°C), the magnitude of this plasticity was generally low compared to two other wide-spread and more geographically-range-restricted con-familial species, *C. capitata* and *C. rosa*. In other words, *B. dorsalis* adults were unable to rapidly heat- or cold-harden to the same extent as the other *Ceratitis* species examined to date. These results suggest a narrower thermal niche in *B. dorsalis* compared to these *Ceratitis* species - in both basal and plastic terms - and suggests that its geographic distribution might be more restricted as a consequence.

The larval stage of fruit flies is the most commonly intercepted life stage, and identification of this stage using traditional morphological methods such as identification keys is difficult. This

study investigated the use of shape analysis, a morphometric method, to identify the third instar larvae of four tephritid species commonly intercepted in fruit destined for export. Larval specimens of laboratory reared *B. dorsalis*, *C. capitata*, *C. rosa* s.s. and *Ceratitis cosyra* (Walker) were used. The mandibles of third instar larvae of all species were dissected out, dehydrated and mounted in Euparal. Images of the mandibles were captured and analysed using Elliptical Fourier Descriptors (in the SHAPE v.1.3 analysis programme). According to the cumulative eigenvalues, the first two Principal Components (PCs) contributed the most (65%) to the shape change. The first PC separates *C. rosa* s.s. and *C. cosyra* from *C. capitata* and *B. dorsalis*. *Ceratitis capitata* and *B. dorsalis* were separated by the second PC. This study showed that morphometrics, in the form of shape analysis of the mandibles, can be used in combination with measurements of the mandibles to distinguish between third instar larvae of *B. dorsalis*, *C. capitata*, *C. rosa* s.s. and *C. cosyra*.

Nutritional stress and population density are some of the factors that can contribute to morphological changes in insects. This study evaluated the effect of four different fruit crops mainly cultivated in Western Cape Province, South Africa: *Prunus persica* (L.) Batsch, (Nectarine), *Prunus domestica* L., (Plum), *Malus domestica* Borkh., (Apple) and *Pyrus communis* L., (Pear) on the wing shape of *B. dorsalis* and *C. capitata*, the dominant fruit fly pest on deciduous fruit in the region. Geometric morphometric tools were used to compare the relative positions of landmarks on the wings of the flies. The results show significant differences in the shape of wings between males and females of both species, indicating sexual dimorphism. The distances between corresponding landmarks among the averaged wings of *B. dorsalis* and *C. capitata* varied highly significantly between individuals that were reared on nectarine, plum, apple and pear. It is as yet unclear how these results translate into fly fitness, but observing significant shape changes resulting from nutritional factors warrant further investigation.

The development, reproduction and survival of *B. dorsalis* and *C. capitata* on main deciduous fruit types cultivated in the Western Cape were studied. For both species, adult emergence was over 90% on all crops, except for *C. capitata* on apple, which was at 84%. The ratio of male:female flies was about 50:50 for both species on all the fruit types. *Bactrocera dorsalis* had a higher net reproductive rate (R_0) on all deciduous fruit tested compared to *C. capitata*. The value of R_0 was the lowest for *C. capitata* on apple and highest on plum. For *B. dorsalis*, R_0 was lowest on nectarine and highest on pear. *Bactrocera dorsalis* adults generally lived longer than those of *C. capitata*, irrespective of the fruit types that they developed from. These results indicate that all the fruit types tested were suitable for both *B. dorsalis* and *C. capitata* to complete their life cycles. The long period of egg production on apple and the high numbers of eggs deposited on pear makes these fruit types ideal bridging hosts for *B. dorsalis* to survive until other hosts become available.

Interspecific competition regulates the distribution and abundance of a number of phytophagous insects. *Ceratitis capitata* is currently the dominant species on deciduous fruit in the Western Cape Province of South Africa. Studies were conducted to quantify adult and larval interactions between *B. dorsalis* and *C. capitata* on four deciduous fruit types (nectarine, plum, pear and apple). When *B. dorsalis* and *C. capitata* adults were evaluated separately, they infested deciduous fruit at more or less the same rates based on the number of pupae reared from the fruit. The only exception was on plum where *B. dorsalis* produced significantly more pupae and consequently adults from the fruit compared to *C. capitata*. When adults of the two species were mixed within a confined space, *Bactrocera dorsalis* was able to out-compete *C. capitata* in most treatments and crops. *Ceratitis capitata* was only able to out-compete *B. dorsalis* on pear. The highest mean numbers of adults of both species emerged from nectarine and plum, with the lowest number emerging from pear. The larvae of *C. capitata* were more successful in completing development than those of *B. dorsalis* when present in mixed ratios as larvae of the two species in plum. *Ceratitis capitata* larvae developed faster than *B. dorsalis* on all deciduous fruit types tested. *Bactrocera dorsalis* larvae were able to complete development more successfully in apple than *C. capitata* in the larval competition experiments. The competition studies between *B. dorsalis* and *C. capitata* demonstrated that on deciduous fruit, competition between the two species would be in favour of *B. dorsalis* at the adult stages (ovipositing females) and, depending on fruit types, in favour of *C. capitata* at the larval stages.

Overall the probability of *B. dorsalis* invading the Western Cape and displacing *C. capitata* in deciduous fruit is bigger than the opposite happening. In case *B. dorsalis* becomes established in the Western Cape, the populations of the pest will probably be reduced to undetectable levels during the winter, with a bloom in the population in summer. As *B. dorsalis* completes more life cycles in Western Cape, it will probably adapt to the local conditions and become a bigger problem for fruit growers. Fruit like apple and pear are not good hosts for *C. capitata*, but might be better hosts for *B. dorsalis*, since *B. dorsalis* deposited a significantly higher number of eggs on pear, lived longer and produced low numbers of eggs over a long time on apple. This could increase the cost of spray programmes, since fruit types with low incidence of spraying could in the presence of *B. dorsalis* require more frequent control interventions.

Opsomming

Bactrocera dorsalis (Hendel) is van Asiese oorsprong, kom in die noordelike en noord-oostelike dele van Suid Afrika voor, maar kom nog nie in die res van Suid Afrika (insluitend die Weskaap) voor nie. Die meeste sagtevrugte word in die Weskaap provinsie van Suid Afrika geproduseer, waarvan 41% van die produksie uitgevoer word. In 2015 het Suid Afrika ongeveer R7 biljoen uit sagtevrugte uitvoere verdien. *Ceratitis capitata* (Wiedemann) en *Ceratitis rosa* s.l. Karsch is die twee vrugtevliespesies met die grootste ekonomiese impak op die sagtevrugtebedryf in Suid Afrika. Die moontlikheid dat *B. dorsalis* na die Weskaap kan versprei en daar vestig is 'n bedreiging vir die sagtevrugtebedryf. Die gebiede waar *Bactrocera dorsalis* in Afrika voorkom het uitgebrei en vergroot oor die laaste 10 jaar soos die vlieg by verskillende klimaatstoestande aangepas het. *Bactrocera dorsalis* was ook in staat om populasies van 'n aantal *Ceratitis* spesies te verdring in Afrika. Die doel van die studie was om die potensiaal van *B. dorsalis* om die Weskaap provinsie van Suid Afrika in te dring en daar te vestig, te bepaal. Die vermoë van *B. dorsalis* om by verskillende temperature aan te pas, die vermoë van die vlieg om sagtevrugte as gasheer te benut en die vermoë om met ander vliespesies te kompeteer om vruggashere is bestudeer. 'n Gebruikersvriendelike basiese identifikasietegniek vir die identifikasie van *B. dorsalis* larwes is ook ontwikkel om te help met die vroeë opsporing van die vliespesie.

'n Gedetailleerde evaluering van die vermoë van vier verskillende lewensstadia van *B. dorsalis* om akute hoë en lae temperature te oorleef is uitgevoer om die hipotese te toets dat sekere eienskappe van die temperatuur nis kon bydra tot die spesie se indringingsvermoë. Die uiterste hoë en lae temperature waarby c. 20% van die *B. dorsalis* populasie sal oorleef, is bepaal deur die vlieë bloot te stel aan -6.5°C en 42.7°C vir 2 uur. Die eiers was die lewensstadium wat die meeste bestand was teen lae en hoë temperature met $44 \pm 2.3\%$ wat die uiterste lae temperature oorleef het en $60 \pm 4.2\%$ wat die uiterste hoë temperatuur oorleef het. Die potensiele vermoë van die volwassenes om beskermingsmeganismes teen uiterste temperature te ontwikkel is getoets oor 'n reeks temperature (blootstelling vir 'n 2 uur tydperk aan 15°C, 10°C en 5°C asook 30°C, 35°C, 37°C en 39°C, met en sonder herstelperiodes). Die resultate toon dat alhoewel *B. dorsalis* wel 'n beskermingsrespons ontwikkel het (byvoorbeeld na blootstelling aan 37°C en 39°C), was die omvang daarvan laer as die van die twee ander wydversreide en meer geografies beperkte *C. capitata* and *C. rosa*. *Bactrocera dorsalis* was nie in staat om 'n hitte-of koue beskermingsrespons te ontwikkel tot dieselfde mate as wat die ander twee spesies dit kon doen nie. Die geografiese verspreiding van *B. dorsalis* mag as gevolg daarvan meer beperk wees.

Vrugtevlieë word meestal in die larwale stadium in vrugte gevind en die identifikasie van larwes deur middel van morfologiese sleutels is moeilik. In hierdie studie is gepoog om die vorm van die mandibels van die derde instar larwes met behulp van morfometriese tegnieke te analiseer en vergelyk. Die mandibels van die vier vrugtevliespesies wat die meeste in uitvoervrugte

gevind word is vergelyk. Die larwes van *B. dorsalis*, *C. capitata*, *C. rosa* s.s. en *Ceratitis cosyra* wat in die laboratorium geteel is, is gebruik. Die mandibels van die derde instar larwes is deur disseksie verwyder en in Euparal op voorwerpglasies monteer. Fotos is van die mandibels geneem en die fotos is geanaliseer deur die “Elliptical Fourier Descriptors” (in die SHAPE v.1.3 analise program) te gebruik. Na aanleiding van die kumulatiewe “eigen”waardes, dra die eerste twee hoofkomponente [Principal Components (PCs)] die meeste (65%) tot die verskille in vorm van die mandibels by. Die eerste PC skei *C. rosa* s.s. en *C. cosyra* van *C. capitata* en *B. dorsalis*. *Ceratitis capitata* en *B. dorsalis* word deur die tweede PC van mekaar geskei. Hierdie studie het getoon dat morfometrie, in die vorm van die analise van die vorm van die mandibels, in kombinasie met afmetings, gebruik kan word om tussen die derde instar larwes van *B. dorsalis*, *C. capitata*, *C. rosa* s.s. en *C. cosyra* te onderskei.

Voedingsstres en populasiedigtheid is faktore wat kan bydra tot verandering in die morfologie van insekte. Hierdie studie het die effek ondersoek wat vier vrugtegewasse wat in die Weskaap Provinsie van Suid Afrika verbou word [*Prunus persica* (L.) Batsch, (Nectarine), *Prunus domestica* L., (Plum), *Malus domestica* Borkh., (Apple) en *Pyrus communis* L., (Pear)] op die vlerkvorm van *B. dorsalis* en *C. capitata* (die dominante vrutevliegplaag op sagtevrugte in die area) het. Geometries-morfometriese metodes is gebruik om die posisies van gekose punte op die vlerke van vlieë te vergelyk. Die resultate toon dat daar betekenisvolle verskille tussen die vlerke van mannetjies en wyfies voorkom by beide spesies, wat op geslagsdimorfisme dui. Die gemiddelde waardes van die afstande tussen die verskillende gekose punte op die vlerke van *B. dorsalis* en *C. capitata* verskil hoogs betekenisvol tussen individue wat hulle lewenssiklus voltooi het op nektarien, pruim, appel en peer. Dit is nog onbekend watter effek hierdie verandering in vlerkvorm as gevolg van die verskille in voedingsfaktore op die lewenskragtigheid van die vlieë het en verdere navorsing is nodig.

Die vermoë van *B. dorsalis* and *C. capitata* om op die hooftipes sagtevrugte wat in die Weskaap verbou word voort te plant en te oorleef, is ondersoek. In die geval van beide spesies het 90% van die papies in volwassenes ontwikkel, met die uitsondering van net 84% van die *C. capitata* papies wat op appel tot volwassenes ontwikkel het. Die verhouding van mannetjies:wyfies was ongeveer 50:50 vir beide spesies op al die vrugtypes. Die netto reprodktiewe koers (R_0) van *Bactrocera dorsalis* was hoër as die van *C. capitata* op al die gewasse. Die R_0 vir *C. capitata* was die laagste op appel en die hoogste op pruim. Die R_0 vir *B. dorsalis* was die laagste op nektarien en die hoogste op peer. Vowasse *Bactrocera dorsalis* vlieë het oor die algemeen langer geleef as *C. capitata*, ongeag van die vrugtype waarop hulle ontwikkel het. Hierdie resultate toon dat al die vrugtypes geskik was vir *B. dorsalis* en *C. capitata* om hulle lewenssiklus op te voltooi. Die hoë aantal eiers wat op peer waargeneem is en die langer tydperk van eierproduksie op appel deur *B. dorsalis* maak hierdie twee vrugsoorte ideale oorbruggingsgashere totdat ander gashere beskikbaar word.

Interspesie kompetisie beheer die verspreiding en getalle van verskeie plantvoedende insekte. *Ceratitis capitata* is op die oomblik die dominante vrugtevliespesie op sagtevrugte in die Weskaap provinsie van Suid Afrika. Studies is uitgevoer om die interaksie tussen die larwes en volwassenes van *B. dorsalis* and *C. capitata* op vier soorte sagtevrugte (nektarien, pruim, peer en appel) te bepaal. In eksperimente waar die interaksie van volwasse *B. dorsalis* en *C. capitata* apart geëvalueer is, was die vlakke van besmetting ongeveer dieselfde, gemeet aan die aantal papies wat voorgekom het. Die enigste uitsondering was dat *B. dorsalis* betekenisvol meer papies en volwassenes as *C. capitata* op pruim voortgebring het. In eksperimente waar volwassenes van die twee spesies in 'n beperkte ruimte geplaas is, was *B. dorsalis* die sterkste kompeteerder in meeste behandelings en op meeste vrugsoorte. *Ceratitis capitata* was slegs op peer die sterkste kompeteerder. By beide spesies het die hoogste gemiddelde aantal volwassenes op nektarien en pruim uitgebroei, met die laagste aantal op peer. Die larwes van *C. capitata* was in staat om meer suksesvol te kompeteer met die van *B. dorsalis* wanneer hulle saam in verskillende verhoudings in pruime geplaas is. Die larwes van *C. capitata* het vinniger as die van *B. dorsalis* ontwikkel op al die soorte vrugte wat getoets is. In die eksperimente waar die larwes van die twee spesies teen mekaar gekompeteer het, kon die larwes van *B. dorsalis* hulle lewenssiklus meer suksesvol op appels voltooi as die van *C. capitata*. Die kompetisie eksperimente tussen *B. dorsalis* en *C. capitata* op sagtevrugte het getoon dat *B. dorsalis* die beter kompeteerder is in die volwasse stadium terwyl die larwes van *C. capitata* beter kompeteerders was op meeste van die vrugsoorte.

In die geheel gesien is die moontlikheid dat *B. dorsalis* se status in die Weskaap sal bereik groter as die kans dat dit nie sal gebeur nie. Wanneer dit gebeur sal die getalle van die populasie waarskynlik onopspoorbaar laag wees in die winter, met 'n opbloeï in getalle in die somermaande. Soos wat die *B. dorsalis* populasie meer lewenssiklusse onder die plaaslike toestande voltooi, sal dit waarskynlik by die plaaslike toestande aanpas en 'n goeter probleem vir produsente word. Kernvrugte soos appel en peer is nie goeie gashere vir *C. capitata* nie, maar mag beter gashere vir *B. dorsalis* wees aangesien *B. dorsalis* betekenisvol hoër getalle eiers op peer gelê het en oor 'n lang tyd lae getalle eiers op appel gelê het. Dit kan die koste van spuitprogramme verhoog, aangesien vrugtipes wat vantevore minder bespuitings nodig gehad het, nou meer dikwels gespuit sal moet word.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- HORTGRO (Mr Matthew Addison and Mr Hugh Campbell) for funding, support and getting permission to keep a colony of *Bactrocera dorsalis* in quarantine in the Western Cape
- My supervisors – Dr Pia Addison, Dr Aruna Manrakhan and Prof John Terblanche for their patience and guidance
- DAFF for awarding me a bursary to fund my tuition
- ARC PPRI Vredenburg for the use of their insect quarantine facility
- Prof. Antoinette Malan, Prof Henk Geertsema, Dr Minette Karsten, Dr Carolien van Zyl, Vernon Steyn and Meshack Magagula of the US and Charmain Theron of the UP for technical assistance/advice/support
- The E-Team at the Plant Quarantine Station (Magrieta Jacobs, Humbulani Ramukhesa and Saadiek Rosenberg) for rearing the flies, technical support and so much more!
- Mrs. Ramukhesa from rural Venda for providing me with infested guavas to start my *Bactrocera dorsalis* colony; Dr Aruna Manrakhan and Mr Rooikie Beck from Citrus Research International (Nelspruit, Mpumalanga, South Africa) for providing me with fruit fly pupae to add fresh genetic material to the colony and showing me how to culture the colony on an artificial diet
- Dr Daleen Stenekamp (Hortgro) for keeping the *Ceratitis capitata* colony
- Ms Gail Jordaan (Dept. Animal Sciences) for help with SAS and Prof DG Nel of the Centre for Statistical Consultation, Stellenbosch University, for statistical advice
- My colleagues at the Plant Quarantine Station for their support
- My husband, Dr PJ Pieterse (my unofficial and overworked “supervisor”) for his unwavering support, mentoring and technical assistance as well as the rest of my family for their patience and support

solī Deo gloria

Preface

This dissertation is presented as a compilation of seven chapters where each chapter stands as an individual unit. Repetition that occurs between chapters was thus unavoidable. Each chapter is introduced separately. Chapters two to four are written according to the style of the journals (*Journal of Applied Entomology*, *Journal of Insect Physiology* and *Zoologischer Anzeiger*) in which the chapters were published. Chapters five and six are written according to the style of *African Entomology*.

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Chapter 2 **The use of shape analysis to differentiate between the mandibles of four economically important tephritid species.**

Chapter 3 **Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)?**

Chapter 4 **The use of Geometric Morphometric Analysis to illustrate the shape change induced by different fruit hosts on the wing shape of *Bactrocera dorsalis* and *Ceratitis capitata* (Diptera: Tephritidae).**

Chapter 5 **Comparative demography of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit.**

Chapter 6 **Interspecific competition between *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit.**

Chapter 7 **General discussion and conclusion**

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Chapter 1

Literature review

Fruit flies belong to the family Tephritidae in the order Diptera and are mostly phytophagous (White & Elson-Harris, 1992). The family Tephritidae has more than 4000 species distributed globally (White & Elson-Harris, 1992). The larvae of about 35% of the species attack fruit that include fruit crops of economic importance (White & Elson-Harris, 1992). Some of these species are among the most destructive pests of fruit and vegetables and are of quarantine importance for the export market (Ekesi *et al.*, 2007, 2016). A few of these frugivorous fruit fly pests are highly polyphagous, attacking a wide range of species in different plant families, and often have overlapping host ranges (White & Elson-Harris, 1992). In environments shared between different polyphagous fruit fly species using similar hosts, competitive interactions are likely to occur. Female choice and larval performance are the two most important factors that determine the suitability of a plant as a host for tephritid species (Jaenike, 1990; Ravigné *et al.*, 2009).

In South Africa, the three indigenous fruit fly species which affect production and export of commercial fruit are: *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* s.l. Karsch and *Ceratitis cosyra* (Walker) (Blomefield *et al.*, 2015). A fourth species of economic importance in South Africa is the exotic pest of Asian origin- *Bactrocera dorsalis* (Hendel) which was recently established in the north and north eastern areas of the country (Grout & Moore, 2015) and is currently considered absent in other parts of South Africa including the Western Cape Province. The Western Cape Province is the largest producer of deciduous fruit in South Africa, with 41% of the deciduous fruit grown in the province being exported. South Africa earned about R7 billion in export revenue from deciduous fruit exports in 2015 (Anonymous, 2015). There is currently a great concern in the deciduous fruit industry of the potential invasion of *B. dorsalis* in the Western Cape Province. *Ceratitis capitata* and *C. rosa* s.l. are economically the most important fruit fly species on deciduous fruit in the Western Cape (Barnes *et al.*, 2007; Manrakhan & Addison 2013), with *C. rosa* s.l. being only more prominent in the milder coastal areas (Barnes & Venter, 2006). *Ceratitis rosa* was recently categorized into two species: *C. rosa* Karsch and *C. quilicii* De Meyer, Mwatawala & Virgilio following morphological, genetic and physiological differences found between these two groups (De Meyer *et al.*, 2016). *Ceratitis quilicii* (previously known as *C. rosa* R2) (De Meyer *et al.*, 2016) is most likely the only one of the two species present in the Western Cape Province of South Africa (Karsten *et al.*, 2016). Based on climatic models, *B. dorsalis* can potentially establish in many parts of the Western Cape Province. (Stephens *et al.* 2007, De Villiers *et al.* 2016).

Ceratitis capitata and *B. dorsalis* are both highly polyphagous and have been reported on more than 100 hosts across the world (Liquido *et al.*, 1990, White & Elson-Harris, 1992, De Meyer *et al.*, 2002, Clarke *et al.*, 2005).

In this review, a background on the taxonomy, identification, biology and ecology of *B. dorsalis* is provided with a focus on host use and invasion capacity (competition and climatic tolerances) by the pest.

1.1 Taxonomic status

The 'new' *Bactrocera* species found attacking fruit in Kenya in 2003 (Lux *et al.*, 2003) was described as *Bactrocera invadens* Drew, Tsuruta and White in 2005 (Drew *et al.*, 2005), a species of Asian origin. More recently, *B. invadens* was synonymized with *Bactrocera dorsalis* (Hendel) following several years of research on the morphology, genetics, chemo-ecology and reproduction of the pest (Schutze *et al.*, 2015b). The findings that led to this taxonomic change are outlined below.

In the description of *B. invadens* (Drew *et al.*, 2005), the species was distinguished from *B. dorsalis* s.s., by the presence of a reddish-brown mesonotum as compared to a black mesonotum in the latter species (Drew & Hancock 1994). *Bactrocera invadens* was classified in the *B. dorsalis* (Hendel) complex of tropical fruit flies (Drew *et al.*, 2005), a complex containing some of the most damaging phytophagous insect pests (Clarke *et al.* 2005). Eleven species closely related to *B. dorsalis* (Hendel) were grouped together in the *B. dorsalis* complex by Hardy (1969). The species complex (consisting of about 75 described species) was comprehensively revised by Drew & Hancock (1994).

The species status of *B. invadens* in the *B. dorsalis* group was first investigated by San José *et al.* (2013) using sequencing of the CO1, EF1 α and PER genes. The latter authors concluded that the species forming part of the *B. dorsalis* clade; *B. invadens*, *B. dorsalis*, *Bactrocera papayae* Drew and Hancock and *Bactrocera philippinensis* Drew and Hancock are polyphyletic and paraphyletic and proposed that the major pest species within the clade represent a single, phenotypically plastic species. In mating compatibility experiments, Bo *et al.* (2014) found that *B. dorsalis* and *B. invadens* mated randomly and had high levels of hybrid viability and survival and as such the authors suggested that *B. invadens* and *B. dorsalis* represented the same biological species. Drew *et al.* (2008) used a combination of external morphological characteristics, molecular analyses, pheromones and host range to investigate the *B. dorsalis* complex group (*B. carambolae*, *B. dorsalis*, *B. occipitalis*, *B. papayae*, *B. philippinensis*, *B. kandiensis* and *B. invadens*) and concluded that there was significant congruence between the members of the group. In 2013, Frey *et al.* (2013) suggested that *B. invadens* should be synonymized with *B. dorsalis* following analyses of the CO1 region and comparisons of single nucleotide polymorphisms (SNPs). The results from chemotaxonomic, chemical ecology and DNA combined in a study by Tan *et al.* (2013) also strongly indicated that *B. philippinensis*, *B. dorsalis* s.s., *B. invadens* and *B. papayae* belong to the same biological species.

Bactrocera invadens Drew, Tsuruta and White was recently synonymized with *B. dorsalis* (Hendel) and with *Bactrocera papayae* Drew & Hancock, *Bactrocera philippinensis* Drew &

Hancock and *Bactrocera carambolae* Drew & Hancock; with *B. dorsalis* (Hendel) accepted as the senior synonym (Schutze *et al.*, 2015 a,b). Drew & Romig (2016) however withdrew *B. papayae* and *B. invadens* from the synonymy. David *et al.* (2017) accepted their taxonomic arguments and included both *B. dorsalis* and *B. invadens* as separate species in their subgeneric key to the *Bactrocera* of India. The decision of Drew & Romig (2016) was condemned by Schutze *et al.* (2017) who proposed that their synonymy stands because of their integrated and peer reviewed approach that led to the synonymization. The synonymization was confirmed by Vaničková *et al.* (2017) after analyzing the chemical epicuticle composition of males and females of the synonymized species. For the purposes of this dissertation, the aforementioned synonymization will be accepted.

1.2 Identification

The larval stages of *B. dorsalis* consist of three instars and were described in detail by White & Elson-Harris (1992), Frias *et al.* (2006, 2008) and Shi *et al.* (2017) and the eggs by Danjuma *et al.* (2015). Adults of *B. dorsalis* can be identified using morphological keys and descriptions (Ekesi & Billah 2007, White & Elson-Harris, 1992; Schutze *et al.*, 2015b). Larval identification of the pest is however more difficult. White & Elson-Harris (1992) included a larval identification key for fruit fly species of economic importance, but it is only valid for final instar larvae and expert knowledge about dipteran larval taxonomy is needed. The key also does not include all species of economic importance in South Africa and Africa (White & Elson-Harris, 1992). DNA barcoding of the CO1 gene can be used to distinguish between fruit fly pests of quarantine importance and other species of lesser concern (Blacket *et al.*, 2012). Larvae of *B. dorsalis* can be identified using DNA barcoding (Khamis *et al.*, 2012; Liu *et al.*, 2011). The limitations of using the CO1 gene for the identification of fruit fly species were highlighted by Armstrong & Ball (2005) and Frey *et al.* (2013). Krosch *et al.* (2012) used the 16S, COI, COII and white eye genes and concluded that *Bactrocera* is paraphyletic and the (*Bactrocera*) *dorsalis* species complex (Drew & Hancock, 1994) is a very recently derived, monophyletic clade. Jiang *et al.* (2014) found that the success of using barcoding as an identification tool for the identification of fruit fly species was reduced by the presence of species complexes. Sequences from the CO1 gene should be combined with other genes and phylogenetic reconstruction to give better clarity in this matter (Khamis *et al.*, 2012). The different molecular methods for identification of tephritids were reviewed by Schutze *et al.* (2015a) & Ekesi *et al.* (2016). The use of DNA barcoding for the identification of insects, including Tephritidae, was reviewed by Jinbo *et al.* (2011).

Shape analysis has been used to separate different tephritid species (using shapes of adult wings and legs) and populations of a number of insect species. Khamis *et al.* (2012) showed that *B. dorsalis* can be morphometrically separated from *Bactrocera correcta* (Bezzi),

Zeugodacus cucurbitae (Coquillett), *Bactrocera oleae* (Rossi) and *Bactrocera zonata* (Saunders) with shape analysis using wing morphology and tibia length. Canal *et al.* (2015) used shape outline analysis of the mandibles in combination with other measurements to distinguish between the third-instar larvae of five morphotypes of the *Anastrepha fraterculus* (Wiedemann) cryptic species complex. Contour shapes of insect structures can be calculated using Elliptic Fourier descriptors (EFDs) in software packages such as SHAPE v.1.3 (Iwata & Ukai, 2002). For larvae of *B. dorsalis*, shape analysis could be explored as a potential species identification tool.

1.3 Distribution

Bactrocera dorsalis invaded and became established in many parts of the world (Vargas *et al.*, 2007; San Jose *et al.*, 2013). *Bactrocera dorsalis* was first recorded in the Asia-Pacific region in 1912 (Shi *et al.*, 2005). Based on its current distribution; *B. dorsalis* can adapt to various climates and can potentially spread to many countries currently free of the pest such as Australia, central America and mainland USA over the next 100 years (Clarke *et al.*, 2005; Aketarawong *et al.*, 2007).

After the first detection of *B. dorsalis* in Kenya (Lux *et al.*, 2003), the pest was detected in many other African countries such as Tanzania, Benin, Uganda, Cameroon, Togo, Senegal, Ghana and Nigeria between 2003 and 2004 (Drew *et al.*, 2005). The species spread to Zimbabwe (Musasa 2013), Sudan (Satti, 2011), Mozambique (Jose *et al.*, 2013; Cugala & Santos, 2013) and Swaziland (Magagula & Nzima, 2017). It was also detected in the Indian Ocean islands off the coast of Africa – Mauritius (eradicated twice, Sookar *et al.*, 2014), Madagascar (EPPO datasheet for *Bactrocera dorsalis*) and the Comoros (Hassani *et al.*, 2016).

The first record of a single specimen of *B. dorsalis* in South Africa was from a methyl eugenol (ME) baited trap in Tshipise in the northernmost part of the Limpopo province in 2007 (Manrakhan *et al.*, 2015). An action plan specific to *B. dorsalis* was compiled in 2008 (Manrakhan *et al.*, 2011, 2012) documenting the recommended response for survey, containment and eradication following a find of *B. dorsalis* in an area having an existing trapping network. The first detection of populations of *B. dorsalis* (more than one specimen in an area of radius 5 km) in the northern parts of South Africa was in 2010 (Manrakhan *et al.*, 2011). An eradication campaign was launched in the affected area and eradication of *B. dorsalis* was confirmed after no finds of the pest in the area for more than 12 weeks after the last fly find (Manrakhan *et al.*, 2011). Incursions of *B. dorsalis* occurred in the previously eradicated areas after a year and were eradicated (Manrakhan *et al.*, 2015). In 2012 and 2013, there were several incursions of *B. dorsalis* in the northern areas (Manrakhan *et al.*, 2015). The pest could not be successfully eradicated in all areas (Manrakhan *et al.*, 2015). *Bactrocera dorsalis* was declared present in the Vhembe district of Limpopo in 2013, where it is under official control. It

was then also found present in other parts of Limpopo (Waterberg, Capricorn, Mopani and Greater Sekhukhune districts), North West (Ngaka Modiri Molema district), KwaZulu-Natal (uThungulu and uMkhanyakude districts) and Mpumalanga (Ehlanzeni district) (IPPC notification 26/6, 2013). In the Northern Cape (Francis Baard and Siyanda districts) the pest was declared as eradicated by the Department of Agriculture, Forestry and Fisheries (DAFF) in 2016 (IPPC notification 28/2, 2016). With a wider spread of the pest, eradication of the pest might not be economically viable and the species might become established as in the case of *B. dorsalis* in the northern parts of South Africa (Manrakhan *et al.*, 2015). Once *B. dorsalis* is established in an area, the pest would have to be managed like other fruit fly pest species. *Bactrocera dorsalis* is considered absent in the Western Cape Province of South Africa (IPPC notification 28/2, 2016). With the Western Cape Province being the biggest producer of deciduous fruit in South Africa (Anonymous, 2015), the presence of *B. dorsalis* in this province is likely to have a serious impact on the export of deciduous fruit.

1.4 Biology

Bactrocera dorsalis was found to reach sexual maturity within seven days (Rwomushana *et al.*, 2009). Females produced most of their eggs between 8 and 22 days after achieving maturity (Rwomushana *et al.*, 2009). Mature females can produce over 1000 eggs in her lifetime, of which 55% will develop to adults (Ekesi *et al.*, 2006). If conditions are suitable, a population *B. dorsalis* can increase by 11% per day and double after six days (Ekesi *et al.*, 2006).

Adults can disperse over long distances (Froerer *et al.*, 2010). They are capable of dispersing between 50 to 100 km and become sexually mature within 7-14 days (Shi *et al.*, 2005; Chou *et al.*, 2012). Male flies are attracted to the naturally occurring phenylpropanoid compound methyl eugenol (ME) (Metcalf, 1975; Shelly, 1994). Methyl eugenol can be found in 450 plant species from 80 families belonging to 38 plant orders (Tan & Nishida, 2012). Adult males form leks to attract females for mating (Shelly, 2001). Adult female *B. dorsalis* display con-specific aggression to defend oviposition spots on mango (Shelly, 1999). Chen *et al.* (2006) found that mainly mated females were present in guava orchards during the day, with the males mostly on the vegetation surrounding the orchards. The population of flies present in the orchards peaked during the late afternoon, moving to the surrounding vegetation during the late afternoon in a diel pattern of movement (Chen *et al.*, 2006).

Bactrocera dorsalis males act as pollinators for orchids in Papua New Guinea, Borneo, Sumatra and Malaysia (Tan *et al.*, 2006; Tan & Nishida, 2007; Tan, 2009). *Bactrocera* spp. were suggested as the sole pollinators of some orchids as in the case of *Bulbophyllum baileyi* F. Muel (Tan *et al.*, 2006). Orchids release floral zingerone and methyl eugenol to attract males to act as pollinators (Tan *et al.*, 2006; Tan, 2009).

1.5 Host plants

Bactrocera dorsalis is an opportunistic, broad range exploiter of fruit pulp, very mobile and exhibit a high reproductive potential (Clarke *et al.*, 2005; Malacrida *et al.*, 2007). Chemical cues, especially from ripe fruit of preferred hosts, influence the choice of oviposition host in gravid females (Jayanthi *et al.*, 2012). According to De Meyer *et al.* (2012), *B. dorsalis* can infest more than 80 host plants. Mango appears to be the primary host (Mwatawala *et al.*, 2004; Ekesi *et al.*, 2006), with guava (*Psidium guajava*; Myrtaceae) (Vargas *et al.*, 2007; Ali *et al.*, 2014; Hussain *et al.*, 2015) and tropical almond (*Terminalia catappa*; Combretaceae) as highly suitable reservoir hosts (Mwatawala *et al.*, 2006, 2009). *Citrus sinensis* (Rutaceae) and avocado (*Persea americana*, Lauraceae) were not favorable hosts for *B. dorsalis* (Mwatawalawa *et al.*, 2006, 2009; Rwomushana *et al.*, 2008), but Goergen *et al.* (2011) found that mandarin orange (*Citrus reticulata*) and sweet orange (*Citrus sinensis*) were good hosts. Mwatawala *et al.* (2009) found that of all citrus fruits tested, *Citrus paradisi* (grapefruit) showed the highest rate of emergence for *B. dorsalis*. The stage of ripeness is important for the acceptability of fruit for oviposition and development of the larvae of *B. dorsalis*. Rwomushana *et al.* (2008) reared *B. dorsalis* from bananas, but Cugala *et al.* (2013) found that green Cavendish dwarf bananas were not a host. Benjamin *et al.* (2012) reported damage to watermelons and vegetable crops such as tomato, peppers and cucumber in Ghana. Adults can use non-host vegetation (such as *Ricinus communis*, *Schinus terebinthifolia*, *Xanthium strumarium* and *Cordyline fruticosa*) as roosting sites at night (McQuate & Vargas, 2007).

Losses suffered due to infestation of fruit with *B. dorsalis* in Africa range between 30% and 80% depending on the fruit, location and season (Mwatawalwa *et al.*, 2006). Losses of 80% in the Sudan (Satti, 2011), 30-60% in Senegal (Diamé *et al.*, 2015) and 36.7% - 92.5% in Mozambique (Jose *et al.*, 2013) have been recorded.

1.5.1 Wing shape analysis

Host fruit has been shown to influence the size and life history parameters of *C. capitata* (Carey, 1984, Krainacker *et al.*, 1987). The shape, size and fitness of fruit flies are influenced by the quality of food available to the larvae as shown by Canato & Zucoloto (1998) in their experiments investigating the effects of carbohydrate ingestion by *C. capitata*.

Landmark based geometric morphometric analysis was used in various studies for taxonomic purposes. Schutze *et al.* (2012) found that while wing size data based on Canonical Variate Analysis (CVA) of fifteen landmarks on the wings failed to discriminate between morphologically similar taxa within the *B. dorsalis* species complex, CVA analysis of wing shape data did discriminate between the species with 93.27% accuracy. Adsavakulchai *et al.* (1998) also found that several species in the *B. dorsalis* complex could be identified with 89.6% accuracy when using discriminant and cluster analysis of wing shape. The reliability of shape variation

rather than variation in size was also confirmed by Gilchrist & Crisafulli (2006) when distinguishing between wild and mass-reared *Bactrocera tryoni* (Froggatt). The current study will establish whether or not the fruit host can influence the wing shape of *C. capitata* and *B. dorsalis*.

1.5.2 Competition

After invading Tahiti in 1996, *B. dorsalis* also displaced other *Bactrocera* species such as *Bactrocera kirki* (Froggatt) and *B. tryoni* (Leblanc *et al.*, 2013). *Bactrocera dorsalis* displaced the established *Ceratitis* species in the lowland areas of the island after invading Hawaii (Keiser *et al.*, 1974). The host plant plays an important role in the ability of *B. dorsalis* to out-compete *C. capitata* in Hawaii, since *C. capitata* was able to persist in the lowlands of Hawaii on coffee (Vargas *et al.*, 1995), an ancestral host of *C. capitata* (Malacrida *et al.*, 1992). *Bactrocera dorsalis* is an aggressive invader, out-competing other Tephritid fruit flies in mango, according to Mwatawala *et al.* (2006), Duyck (2006) and Ekesi *et al.* (2009). After being detected in the northern region of Swaziland in January 2013 for the first time, *B. dorsalis* is now the dominant species over *C. capitata*, *C. cosyra* and *C. breinii*, which were the most abundant fruit fly species detected on mango before the invasion of *B. dorsalis* (Magagula & Nzima, 2017). Migani *et al.* (2014) showed that *B. dorsalis* females are more prone to accept new and less preferred hosts, because of its high and continuous egg production. Duyck *et al.* (2007) showed that some invasive species can co-exist through the mechanism of climatic niche partitioning. Ekesi *et al.* (2009) showed that in co-infestations of mango fruits, *C. cosyra* larvae were negatively affected by *B. invadens*, because it competes more effectively for the available resources. Mwatawala *et al.* (2006) found that *B. dorsalis* was the dominant species reared from a range of tropical crops in Tanzania. Exploitative competition by tephritid larvae in fruit can lead to lower pupal weight and impact negatively on larval survival (Duyck *et al.*, 2008), with the resulting influence on the net reproductive rate. This link between fruit type and net reproductive rate has been illustrated by Krainacker *et al.* (1987) for *C. capitata* and Ekesi *et al.* (2006) for *B. dorsalis*. In this thesis, the ability of *B. dorsalis* to compete with the widespread *C. capitata* in deciduous fruit will be investigated.

1.6 Fruit fly management

Populations of fruit flies can be monitored by using male lures, food-based attractants and pheromones mainly in bucket type traps (Shelly *et al.*, 2004; Manrakhan *et al.*, 2017). The traps used to attract *B. dorsalis* (and fruit flies in general) are normally yellow in color (Wu *et al.*, 2007). Traps containing methyl eugenol are highly attractive to male *B. dorsalis* (Tan *et al.*, 2006, Vargas *et al.*, 2000). Piñero *et al.* (2017) found that beer waste with added ammonium acetate could be used by resource-poor farmers as food bait for various fruit flies, including *B. dorsalis*. *Bactrocera dorsalis* can be controlled by sprays of insecticides (Hsu *et al.*, 2004,

2006), with integrated pest management (IPM) (Verghese *et al.*, 2004; Jin *et al.*, 2011) and area-wide methods such as protein bait sprays, male annihilation (MAT blocks) (Grout & Stephen 2013), SIT (Aketarawong *et al.*, 2011) and natural enemies such as *Fopius* sp. (Vargas *et al.*, 2007, 2012). Effective control of *B. dorsalis* in mango orchards in India was exercised by using a combination of orchard sanitation, inter-tree ploughing and raking and insecticide cover sprays (Verghese *et al.*, 2004). Seewooruthun *et al.* (1997) reported the results of using bait application and male annihilation combined with orchard sanitation and cover sprays with insecticides to contain the spread of *B. dorsalis* in Mauritius, resulting in the eradication of the species on the island in 2000 (Sookar *et al.*, 2006). Plywood blocks impregnated with methyl eugenol as attractant and malathion as insecticide was the most effective when evaluated against other material such as sponge, cotton and white wood (Sidahmed *et al.*, 2014). Diamé *et al.* (2015) described the use of *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae, weaver ant) as biological control agents of fruit flies in mango orchards. The oviposition and mating behavior of *B. dorsalis* and *C. capitata* will be recorded on the deciduous fruit tested in this study. This will give an indication of the time of day and fruit type that are preferred for oviposition by the two fruit fly species. This information can contribute to improving management strategies in deciduous fruit orchards.

1.7 Phytosanitary status and treatments

The European Plant Protection Organization includes *B. dorsalis* in their A1 quarantine list (<http://www.eppo.int/QUARANTINE/listA1.htm>) and quarantine measures are implemented by many countries to prevent entry of this pest. The quarantine importance of this pest is illustrated by Cugala & Santos (2013), stating that the temporary closure of the South African market for three weeks in October 2008 resulted in the loss of about 2.5 million US dollars by Mozambique. In South Africa, a national surveillance programme for the detection of exotic fruit flies was started in April 2006 (Barnes & Venter, 2006). This surveillance programme was intensified specifically targeting *B. dorsalis* in 2008 after its detection in Zambia, Mozambique and Namibia (Manrakhan *et al.*, 2011). The surveillance programme is being carried out by the National Plant Protection Organisation of South Africa (NPPOZA) in collaboration with fruit industries. Surveillance is carried out through trapping in some production areas, in major towns and at points of entry (border areas, border posts, ports and airports) according to a national action plan (Manrakhan *et al.*, 2012).

Fruit for export from South Africa are already subject to various heat or cold treatments designed to control Lepidoptera pest species (Dohino *et al.*, 2016). The temperatures used in these treatments are low enough to kill Tephritidae (Grout *et al.*, 2011; Ware *et al.*, 2012). The use of hot-water treatments to control tephritid larvae in export fruit has been researched in Africa (Self *et al.*, 2012) but is not yet implemented as a method for quarantine disinfestation of

fruit exported from South Africa. The viability of heat treated fruit fly eggs can be tested using bioluminescent adenosine triphosphate (ATP) assays (Kamiji & Kadoi, 2017). Digitized X-ray images of fruit can be used to detect infestations of *B. dorsalis* in fruit (Yang *et al.*, 2006). Infested fruit can be detected between 3-6 days after infestation and the method was tested to be effective in tomato, orange, apple, pear and peach fruit. The temperature tolerances of the different life stages of *B. dorsalis* will be investigated in this study to determine the stage most vulnerable to high or low temperatures.

1.8 Invasive ability

Sakai *et al.* (2001) describes phenotypic plasticity, competitive ability and the ability to spread as some of the characteristics that species need to colonize, establish and invade new areas. *Bactrocera dorsalis* is known to be a large, dominant fly, able to out-compete *Ceratitis* species in Africa (Duyck *et al.*, 2006; Mwatawala *et al.*, 2006; Ekesi *et al.*, 2009). After invading Tahiti in 1996, *B. dorsalis* displaced *B. kirki* and *B. tryoni* as the most prominent fruit fly pest (Leblanc *et al.*, 2013). According to Geurts *et al.* (2014) the abundance of *B. dorsalis* is influenced by the presence of mango and guava, the preferred host species (Mwatawala *et al.*, 2006; Ali *et al.*, 2014) and the presence of its direct competitor *C. rosa*. *Ceratitis rosa* and *B. dorsalis* were reared from peach fruit with *C. rosa* being more abundant at higher altitudes (Geurts *et al.*, 2014). Han *et al.* (2011) found that only a small percentage of *B. dorsalis* pupae were able to survive the low winter temperatures in the Hubai Province in China; with no adult and larval survivors. Lower elevation above sea level and high temperatures also influenced the population levels of *B. dorsalis* (Vayssieres *et al.*, 2005; Ekesi *et al.*, 2006; Geurts *et al.*, 2014). According to De Meyer *et al.* (2010), *B. dorsalis* prefers hot and humid environments, but can survive dry seasons. *Bactrocera dorsalis* was accidentally introduced onto Hawaii in 1946, causing the population of *C. capitata* to drop at lower elevations, but populations of *C. capitata* stayed abundant in many upland areas (Bess, 1953). According to Clarke *et al.* (2005) differences in life history traits might also influence the successful invasion of fruit flies.

Currently CLIMEX-based models are available (Stephens *et al.*, 2007; Sridhar *et al.*, 2014) and being developed further, but they are one of several potential modeling approaches (e.g. the ecological niche modeling technique, (De Meyer *et al.*, 2008); each with their own advantages and disadvantages. Jayanthi & Vergese (2011) identified the availability of immature guava fruits as the most influential phenological factor in predicting the increase in population numbers of *B. dorsalis*. Combining weather variables with host availability did not increase the predictability of population increases. They proposed using the availability of immature guava fruit as phenological indicator to predict changes in the *B. dorsalis* population because it produces a linear correlation with trap catches.

When using the CLIMEX model developed by Stephens *et al.* (2007), which was designed to predict the spread of *B. dorsalis*, areas further south in South Africa were predicted to be suitable for *B. dorsalis*. These areas have a warm, temperate climate and are humid with hot summers. A mean temperature of 25°C and mean average rainfall of 3000 mm increased the abundance of *B. dorsalis* in traps in the Comores (Hassani *et al.*, 2016). *Bactrocera dorsalis* was also observed in areas of West Africa, the Sudan and Zambia, where the climate has longer dry periods and hot conditions prevail during part of the year (De Meyer *et al.*, 2010). According to Hill & Terblanche (2014) and De Villiers *et al.* (2016), *B. dorsalis* will be able to establish in the Western Cape Province of South Africa based on the models they applied. Hill & Terblanche (2014) also stated that physiological traits and adaptation to new host plants can influence the ability of *B. dorsalis* to establish in areas predicted to be suitable. Climate change might not increase the possibility of *B. dorsalis* to establish in the south-western Cape (Hill *et al.*, 2016), but the thermal adaptability of the invasive fly will influence its ability to invade a new environment.

1.8.1 Thermal Plasticity

The temperature tolerances for *B. dorsalis* adults and immature stages have been explored previously (e.g. Vargas *et al.*, 1996, 1997; Ekesi *et al.*, 2006; Chen *et al.*, 2006; Rwomushana *et al.*, 2008; Ye & Liu, 2005). Survival, reproduction and longevity under different temperature regimes are important factors to consider when fruit flies are accidentally introduced into new areas and likely form a critical part of the invasion process (i.e. overcoming a key population establishment barrier). For example, extreme high temperatures (minimum temperature of 24°C and maximum temperature of 35°C) reduced the population growth of *C. capitata*, *B. dorsalis* and *Z. cucurbitae* and was more limiting for establishment than lower temperatures (Vargas *et al.*, 2000). The ability to rapidly alter tolerance and survival through phenotypic plasticity (physiological adjustments) may also improve the survival capacity of a species introduced to a novel environment, as illustrated for Tephritidae by Nyamukondiwa *et al.* (2010) with *C. capitata* relative to *C. rosa*. The ability to survive low temperatures was markedly improved after pre-treating *C. capitata* and *C. rosa* for 2 h at 5°C and 10°C. However, the importance of plastic hardening responses across a wider range of Tephritidae has generally been poorly examined (Nyamukondiwa *et al.*, 2010). Hu *et al.* (2014) showed that *B. dorsalis* possess a heat hardening response at 35°C, 37°C, 39°C and 41°C; and has a wider distribution range as well as a higher thermal plasticity than *B. correcta*. However, *B. dorsalis* thermal responses relative to other economically-important Tephritidae in South Africa has been poorly explored to date and is essential to understand the relative risks of establishment and population persistence if accidentally introduced into this fruit growing region.

1.9 Rearing of colonies

For laboratory-based experimentation to take place on insects, as is the case in this study, it is relevant to briefly discuss various rearing methods. Fruit flies are usually reared on a solid diet modified from the diet described by Tanaka *et al.* (1969). The process of rearing various tephritid fruit flies in an African context are described in detail by Ekesi & Mohamed (2011) and Barnes *et al.* (2007). The use of “diet balls” containing a carrot-based diet to rear *B. invadens* on a small scale was described by Ekesi *et al.* (2007). Liquid diets for the rearing of fruit flies were investigated because of the cost, problems with disposal of used diet and contamination of some batches of bran with pesticides as well as variation in bran quality (Khan *et al.*, 2011). A liquid rearing diet for small scale rearing of *B. cucurbitae* was developed by Chang *et al.* (2004) and adapted by Chang *et al.* (2006) for the mass rearing of *B. dorsalis*. Ekesi & Mohamed (2011) compared various solid and liquid diets for mass rearing fruit flies and concluded that *B. invadens* can be successfully reared on a liquid diet. Chang & Vargas (2007) showed that the addition of wheat germ oil to liquid fruit fly rearing diets improved fly quality. Khan *et al.* (2011) developed a liquid larval diet for the mass rearing of *B. dorsalis* to save costs. They concluded that their liquid diet containing soy bran and soy protein was promising to use as a cheaper replacement for the liquid diet containing essential amino acids as described by Chang *et al.* (2004). The use of bananas to rear *B. dorsalis* was described by Jayanthi & Verghese (2002) as an easy and economical way to rear the flies.

The importance of protein in the larval and adult diet was investigated by Shelly *et al.* (2005) indicating that males starved of protein mated less frequently (less 5% of the total number of matings) than males who received protein in their diet.

The optimal rearing temperature for *B. dorsalis* is 28°C according to Rwomushana *et al.* (2008) and between 26°C and 28°C according to Ekesi & Mohamed (2011). Arakaki *et al.* (1984) observed mating at a rearing temperature of 27°C from 11 days after emerging and 50% of the population mated after 29 days. Ekesi *et al.* (2006) reported oviposition from 7.1 days and Vargas *et al.* (1996) reported oviposition from 5.3-5.7 days from emergence at 28°C. The number of eggs per female peaked between 10-15 days after emergence (Ekesi *et al.*, 2006). Flies for this study was reared on the artificial larval rearing medium as in Barnes *et al.* (2007) with the addition of 100g carrot powder per kg of mix adapted from Ekesi & Mohamed (2011). This combination was chosen because this study needed both *C. capitata* and *B. dorsalis* to be reared in colonies, without having to prepare a different rearing medium for each species.

1.10 Aim and objectives

The overall aim of the research was to determine the invasive potential of *B. dorsalis* on the deciduous crops grown in the Western Cape of South Africa. Firstly we have to be sure of the identity of the fruit fly species we encounter in fruit samples. A low cost, practical and effective

method of identifying the larvae of four tephritid species of economic importance was deemed important to support quarantine inspections for import or export of fruit consignments from South Africa. The mouthhooks of the third instar larvae of *Bactrocera dorsalis*, *Ceratitis capitata*, *Ceratitis rosa* s.s. and *Ceratitis cosyra* were investigated, to determine whether this can be used as a characteristic to accurately distinguish between these larvae.

Due to *B. dorsalis* being predominantly a tropical pest, the effect of high and low temperatures on survival of adult flies are of interest, since the Western Cape has a Mediterranean climate. The effect of deciduous fruit (the predominant fruit type in the Western Cape) on adult characteristics and demography would also help in forecasting the invasion potential of *B. dorsalis*. Finally, it would be important to quantify the effect of competitors such as other fruit fly pests on reproduction, survival and development of *B. dorsalis* in order to predict the speed and degree of potential invasion by *B. dorsalis*.

The study consists of five chapters to address the three questions above. Each objective was written as an individual chapter and was compiled as separate papers for publication in peer-reviewed scientific journals, and some repetition is therefore unavoidable. They are as follows:

- The use of shape analysis to differentiate between the mandibles of four economically important tephritid species.
- Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)?
- The use of Geometric Morphometric Analysis to illustrate the shape change induced by different fruit hosts on the wing shape of *Bactrocera dorsalis* and *Ceratitis capitata* (Diptera: Tephritidae).
- Demographic parameters of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit.
- Interspecific competition between *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit.

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Chapter 2

The use of shape analysis to differentiate between the mandibles of four economically important Tephritid species

Published as: PIETERSE, W., MANRAKHAN, A., RAMUKHESA, H.R., ROSENBERG, S.M. & ADDISON, P. 2017. The use of shape analysis to differentiate between the mandibles of four economically important tephritid species. *Journal of Applied Entomology* **141**(6): 450-457.

2.1 Introduction

Fruit flies are destructive pests of fruit and vegetables and are of quarantine importance for the export market (White and Elson-Harris 1992). In South Africa, the three indigenous fruit fly species which affect production and export of commercial fruit are: *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* s.l. Karsch and *Ceratitis cosyra* (Walker) (Blomefield et al. 2015). A fourth species of economic importance in South Africa is the exotic pest of Asian origin- *Bactrocera dorsalis* (Hendel) (Grout and Moore 2015). *Bactrocera dorsalis* was recently declared present in the northern and north eastern parts of South Africa (Manrakhan et al. 2015) but is still absent in other areas of the country including the Western Cape Province.

The Western Cape Province is the largest producer of deciduous fruit in South Africa, exporting 41% of the deciduous fruit grown in the province. South Africa earned about R7 billion in export revenue from deciduous fruit exports in 2015 (Anonymous 2015). All fruit that is exported from South Africa must be inspected for the presence of the organisms listed on the import conditions of the importing country. Various Tephritidae are listed on the import conditions of the countries South Africa exports fruit to, with *B. dorsalis* included on all lists. Other tephritid species present in South Africa are limited in their host use and the hosts being used are not commercially produced for export in South Africa.

The larval stage of fruit flies is the stage that is normally detected in samples inspected for quarantine purposes. Identification of larvae in the laboratory of the Department of Agriculture, Forestry and Fisheries (DAFF) at the Plant Quarantine Station in Stellenbosch, Western Cape Province, is currently done using molecular analysis or rearing of flies to adulthood. Molecular analysis is costly and rearing of flies to adulthood takes time, so a method for the identification of larvae using morphological methods was required.

Adult Tephritidae can be identified using morphological keys (Ekesi and Billah 2007, White and Elson-Harris 1992). White and Elson-Harris (1992) included a larval identification key, but some important pest species in South Africa were omitted. Current limitations with keys are that those that exist are not comprehensive and have been based on too few taxa to be reliable. Larval identification is difficult and knowledge of the taxonomy and morphology of larvae is important for their correct identification (Frías et al. 2008).

The larvae of some *Ceratitis* and *Bactrocera* species including *C. capitata*, *C. rosa* and *B. dorsalis* were described in detail by White and Elson-Harris (1992), Carroll (1998), Frías et al. (2006, 2008) and Steck and Ekesi (2015). Most publications do not include measurements of the cephalopharyngeal skeleton and use Scanning Electron Microscope (SEM) photographs to illustrate certain characteristics. Since the use of SEM photography is not widely available, this is only useful for taxonomists in well-equipped laboratories.

DNA barcoding of the CO1 gene can be used to distinguish among fruit fly pests of quarantine importance and other species of lesser concern (Blacket et al. 2012). Larvae of *B. dorsalis* can be identified using DNA barcoding (Khamis et al. 2012, Liu et al. 2011). The limitations of using the CO1 gene for the identification of fruit fly species were discussed by Armstrong and Ball (2005), Frey et al. (2013) and Schutze et al. (2012, 2015), who highlighted the problems with accurate molecular identification of some species because of recent genetic differentiation in the Tephritidae.

Shape analysis has been used to separate different tephritid species (using shapes of adult wings and legs) and populations of a number of insect species. Khamis *et al.* (2012) showed that *B. dorsalis* can be morphometrically separated from *Bactrocera correcta* (Bezzi), *B. cucurbitae* (Coquillett), *B. oleae* (Rossi) and *B. zonata* (Saunders) with shape analysis using wing morphology and tibia length. Yee et al. (2009) used canonical variate analysis of measurements of nine body parts as well as wing shape analysis to discriminate between two *Rhagoletis* species: *R. pomonella* (Walsh) and *R. zephyria* Snow. Mahinay et al. (2014) used shape analysis to describe mandible shape variation in the rice leaf folder, *Marasmia patnalis* Bradley (Lepidoptera: Pyralidae), feeding on different rice varieties. Shape analysis was also used to determine intraspecific variation of male genitalia in *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) by Rentel 2013; variation in male and female beetle genitalia by Polihronakis 2006; male genitalia in *Bombus* (Hymenoptera: Apidae) by Özenirler and Aytekin (2015) and the shape of larval mandibles of *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) by Tabugu et al. (2012). Canal et al. (2015) used shape outline analysis of the mandibles in combination with other measurements to distinguish between the third-instar larvae of five morphotypes of the *Anastrepha fraterculus* (Wiedemann) cryptic species complex. Contour shapes of insect structures can be calculated using Elliptic Fourier descriptors (EFDs) in software packages such as SHAPE v.1.3. The contour shapes are then converted to principal components for a principal components analysis and visualization of shape changes (Iwata and Ukai 2002).

A low cost, practical and effective method of identifying the larvae of four tephritid species of economic importance was deemed important to support quarantine inspections before export of fruit consignments from South Africa. We used SHAPE v.1.3 (Iwata and Ukai 2002) to compare the mandibles of the larvae of four fruit fly pests of commercial fruit in South Africa: *B. dorsalis*,

C. capitata, *C. rosa* s.s. & *C. cosyra*. We also measured and analysed various length measurements of the mandibles to supplement the shape data. This was done to assess the accuracy of using this method to differentiate between larvae of these four species, and to determine which character would be the best to use for this purpose.

2.2 Materials and methods

2.2.1 Preparation of slides

Larval specimens of three *Ceratitis* species, *C. capitata*, *C. rosa* s.s. and *C. cosyra*, were obtained from colonies held at Citrus Research International (CRI) in Nelspruit, Mpumalanga Province, South Africa. Colonies of the three *Ceratitis* species had been maintained at CRI since 1999 and they are regularly refreshed by addition of wild males every 2 years. *Bactrocera dorsalis* larvae from a colony which originated from infested fruit in Vhembe District, Limpopo Province, and kept in quarantine in Stellenbosch since 2014, were used. For all four species, third instar larvae were used. The first and second instar larvae were not included, because they might still possess a secondary tooth on the mandible which might not be present in the third instar (White and Elson-Harris 1992). Larvae were killed by immersing them in hot water and were then preserved in 70% ethanol. Thereafter, the heads of the larvae were cut off and cleared by heating the heads in 10% NaOH. The cephalopharyngeal skeletons (fig. 1) of the larvae of all species were dissected out, dehydrated with alcohol (70-100%) and mounted in Euparal on glass slides. Images of the mandibles were captured using a Nikon Eclipse 80i compound microscope fitted with an Optica digital camera using Optica Vision Pro ver. 2.7 software (fig. 2). Images of 22 *C. capitata* larvae, 21 *C. rosa* s.s. larvae, 21 *C. cosyra* larvae and 36 *B. dorsalis* larvae were captured, and edited using PhotolImpact 6 (fig. 2). Measurements were taken of the distance between the ventral apodeme and the apical tooth (a), the dorsal apodeme and the ventral apodeme (b), and of the ventral angle between the apical tooth and the ventral apodeme (c) (fig. 3). Measurements were taken using the Optica Vision Pro software and recorded in μm (distances) and degrees (angles).

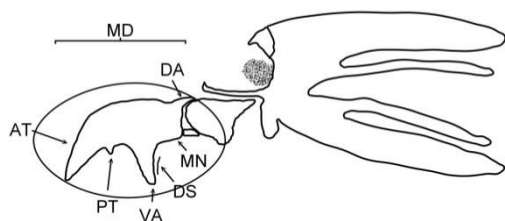


Figure 1. Cephalopharyngeal skeleton of 3rd instar larva of a fruit fly species, lateral view. Region of interest circled: AT = Apical Tooth; DA = Dorsal Apodeme; DS = Dental Sclerite; MD = Mandible; MN = Mandibular Neck; PT = Preapical Tooth; VA = Ventral Apodeme (FROM: FRÍAS et al. 2006)

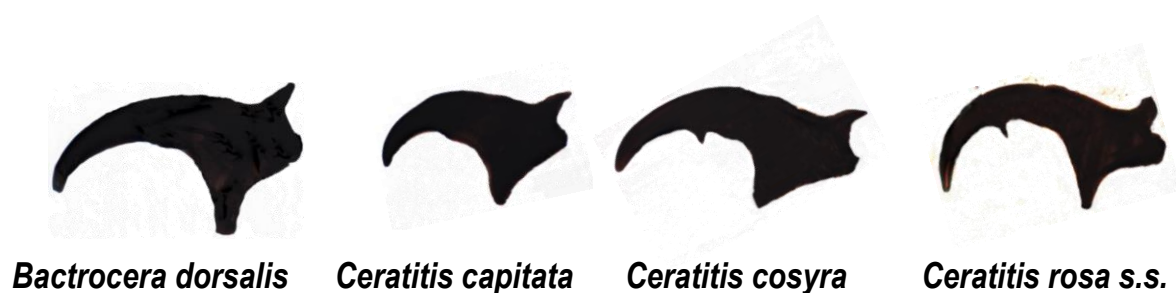


Figure 2. Outlines of the mandible images of *Ceratitis capitata*, *C. rosa s.s.*, *C. cosyra* and *Bactrocera dorsalis*.

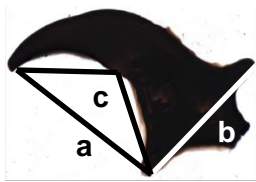


Figure 3. Image of a typical tephritid mandible indicating the areas measured.

2.2.2 SHAPE and data analysis

SHAPE analysis followed the procedure outlined in Gilligan and Wenzel (2008). SHAPE v.1.3 was used, which is composed of three modules: 1) Chaincoder, 2) Chc2Nef, and 3) PrinComp. The first module, Chaincoder, converts the outline of the mandible into a chaincode which is then used by the second module, Chc2Nef, to calculate normalized EFDs (Elliptical Fourier descriptors) based on the first harmonics (Iwata and Ukai 2002). The third module, PrinComp, is used to conduct a principle components (PC) analysis of the coefficients of the Elliptic Fourier descriptors (EFDs) (Iwata and Ukai 2002). PrinComp provides a summary and visualization of the variation in shape for each PC axis.

The eigenvalues for each axis were assessed to determine the proportion of variance explained by each principle component axis. Graphs of the Principal Component Analysis (PCA) were produced using STATISTICA 64 (v. 13, Dell Inc., Tulsa, USA). A one-way ANOVA with multiple variables and discriminant analysis with classification functions of the effective principal components was carried out to determine differences in outlines and measurements of the mandibles between the fruit fly species studied (STATISTICA 64 (v. 13, Dell Inc., Tulsa, USA).

2.2.3 Validation testing

Blind testing of the new morphological technique developed in this study (Analysis of shape variation followed by measurements and identification of features of the larval mouthparts of fruit flies) was carried out. A total of 16 slides which contained the mounted mandibles of the third instar larvae of the four fruit fly species studied here (four slides per species) were given to

three novel users for analysis. The novel users were familiar with the use of a compound microscope and the Optica Vision Pro ver. 2.7 software. A schematic diagram showing the features of the mandibles of the third instar larvae (fig 2.) and a copy of the measurements in Table 4 were provided to each of the users. The 16 slides presented separately to the three users were labelled using codes 1-16 with none of the species following any particular order. The identities of the slides were only known to the author WP. The percentage of positive identification for each species was determined by counting the number of slides correctly identified of the by all users divided by the number of slides available for each species.

2.3 Results and Discussion

According to the cumulative eigenvalues of the co-variance matrix calculated with SHAPE, the first two PCs contributed most (65%) to the shape change (Table 1).

Table 1. Eigenvalues and proportions of the first five Principal Components calculated using SHAPE analysis.

	Eigenvalue	Proportion (%)	Cumulative (%)
PC 1	0.006271136	48.6961	48.6961
PC 2	0.002140254	16.6193	65.3154
PC 3	0.001364851	10.5982	75.9136
PC 4	0.000770788	5.9853	81.8989
PC 5	0.000535380	4.1573	86.0562

Re-constructed shape contours from PrinComp allowed for visualization of the shape variation as described by individual components (fig. 4).

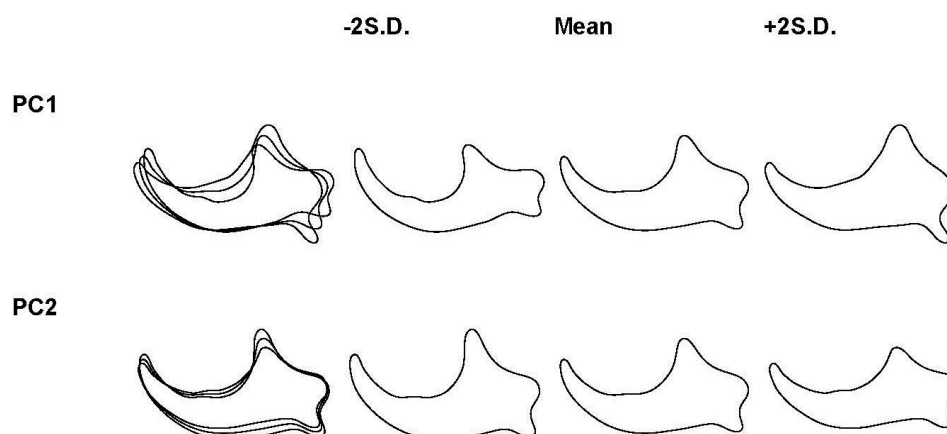


Figure 4. Shape variation of the mandibles of *B. dorsalis*, *C. capitata*, *C. cosyra* and *C. rosa* s.s. as described by the first two Principal Components.

The first PC indicates the change in the angle and distance between the tip of the apical tooth and the ventral apodeme. The second PC indicates the difference in the width between the dorsal apodeme and the ventral apodeme.

According to the bi-plot of the first two PCs from the PCA (fig. 5), the first PC mostly separates all the species. *Ceratitis capitata* and *B. dorsalis* can be separated with greater confidence from *C. rosa* and *C. cosyra*, but there is an overlap between the outlines of *C. rosa* s.s and *C. cosyra*, which makes differentiation between these two species less distinct.

There are statistically significant differences between the outlines of the mandibles of the four species (Wilks' λ =.06080, approx F (21,379)=29.853 p<0.0001). According to the results of the discriminant function analysis, *Bactrocera* can be distinguished from all the other species in most instances (Table 2); with only one sample categorized as *C. capitata*. The identification of *Ceratitis capitata*, *C. cosyra* and *C. rosa* s.s. would also be correct in between 95% and 96% of the cases.

Table 2. Percentages of the third instar larvae of *Bactrocera dorsalis*, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis rosa* s.s. that will be identified correctly when using the discriminant function analysis of the significant Principal Components.

species	Percent correct	<i>Bactrocera dorsalis</i>	<i>Ceratitis capitata</i>	<i>Ceratitis cosyra</i>	<i>Ceratitis rosa</i> s.s.
<i>Bactrocera dorsalis</i>	98.24561	56	1	0	0
<i>Ceratitis capitata</i>	96.87500	1	31	0	0
<i>Ceratitis cosyra</i>	95.65218	1	0	22	0
<i>Ceratitis rosa</i> s.s.	96.66666	1	0	0	29

Ceratitis capitata can be separated morphologically from *C. cosyra* and *C. rosa* s.s. by the absence of a secondary tooth on the mandible, and from *B. dorsalis* by the smaller distance between the ventral apodeme and the apical tooth (a). The secondary tooth on the mandibles of *C. rosa* and *C. cosyra* is present in the third instar larvae and is easy to see using a compound microscope with 10x magnification. It is absent from the mandibles of *B. dorsalis* and *C. capitata* (White and Elson-Harris 1992). Steck and Ekesi (2015) indicated that a poorly developed secondary tooth is often present on the mandibles of *C. capitata* larvae, which can only be visible using a scanning electron microscope.

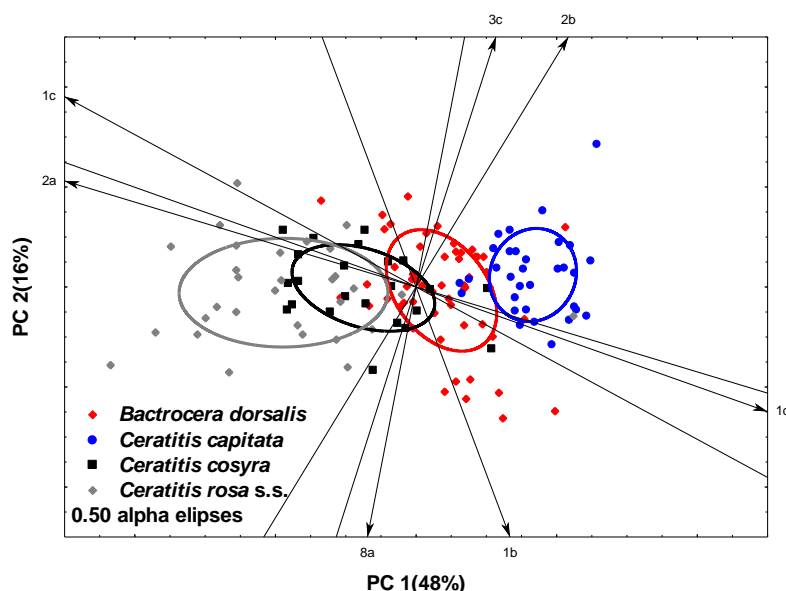


Figure 5. Biplot of the first two Principal Components of the contour shapes of the mandibles of third instar larvae of *B. dorsalis*, *C. capitata*, *C. cosyra* and *C. rosa* s.s. (PC1 measured the angle between the tip of the apical Tooth and the Ventral Apodeme and PC2 measured the distance between the top of the mandible and the Ventral Apodeme). R^2 Threshold =0.5. The arrows indicate the main directions of change in the co-variance matrix.

The distances a and b and angle c (fig. 3) were further analyzed in an effort to separate *C. rosa* s.s. and *C. cosyra* more conclusively. The areas measured were determined by the directions where most of the change was indicated according to the first Principal Component (PC) after re-constructing the shape contours from PrinComp (fig. 4). There were statistically significant differences in the measurements of distances a, b and angle c between the species (Wilks' λ =.03715, F approx. 54.352, p <0.0001). A bi-plot of the co-variance matrix of measurements a, b and c (fig. 6) illustrates that *C. rosa* and *C. cosyra* could now be significantly separated using these measurements, with overlapping measurements for *C. capitata* and *C. cosyra*.

According to the results of the discriminant function analysis, *Bactrocera dorsalis* could be distinguished from all the other species in most instances (Table 3). *Ceratitis capitata* and *C. cosyra* could only be separated with a 63% certainty. The absence of the secondary tooth on the mandibles of *C. capitata* can additionally be used as a morphological characteristic to distinguish between *C. capitata* and *C. cosyra*. *C. cosyra* was categorized as *C. rosa* s.s. in only two instances (Table 3).

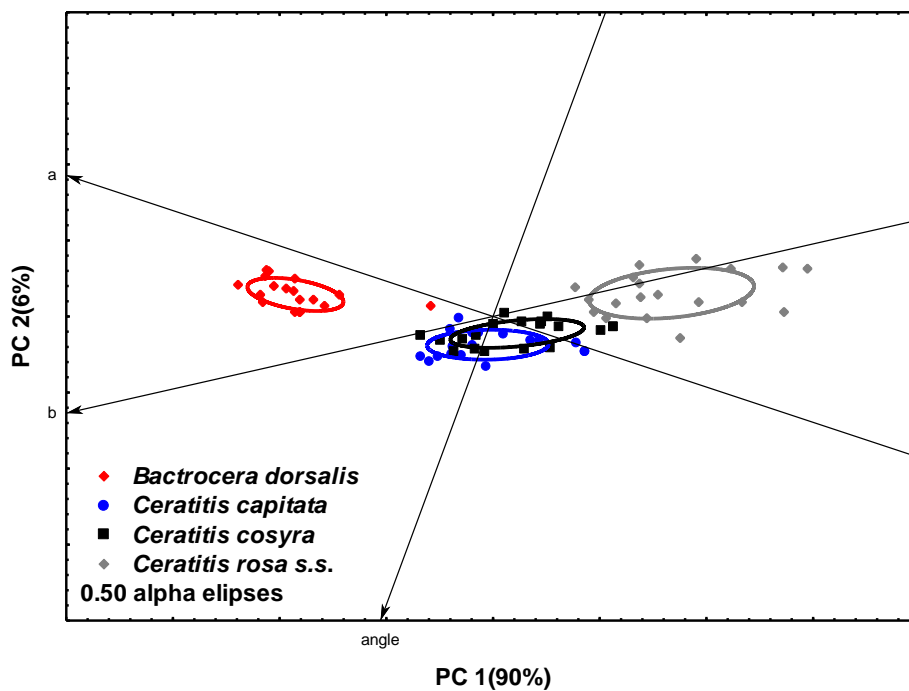


Figure 6. Biplot of the first two Principal Components of measurements a, b and c of the mandibles of third instar larvae of *Bactrocera dorsalis*, *C. capitata*, *C. cosyra* and *C. rosa s.s.* R^2 Threshold = 0.5.

Table 3. Percentages of the third instar larvae of *Bactrocera dorsalis*, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis rosa s.s.* that will be identified correctly when using the discriminant function analysis of measurements a, b and c combined.

species	Percent correct	<i>Bactrocera dorsalis</i>	<i>Ceratitis capitata</i>	<i>Ceratitis cosyra</i>	<i>Ceratitis rosa s.s.</i>
<i>Bactrocera dorsalis</i>	94.73684	18	0	1	0
<i>Ceratitis capitata</i>	63.15789	0	12	7	0
<i>Ceratitis cosyra</i>	63.15789	0	7	12	0
<i>Ceratitis rosa s.s.</i>	89.47369	0	0	2	17

Table 4. Minimum, maximum and means of distances between the ventral apodeme and the apical tooth (a) and from the dorsal apodeme to the ventral apodeme (b), and the ventral angle between the dorsal apodeme and the ventral apodeme (c) of *Bactrocera dorsalis*, *Ceratitis capitata*, *C. cosyra* and *C. rosa s.s.* * = corresponding values from Steck and Ekesi (2015).

	a (mm)			b (mm)			c (degrees)		
	min	max	mean	min	max	mean	min	max	mean
<i>Bactrocera dorsalis</i>	0.15	0.19	0.17	0.14	0.16	0.15	100	110	105
<i>Ceratitis capitata</i>	0.13 (0.14)*	0.15 (0.15)*	0.14	0.13 (0.14)*	0.15 (0.16)*	0.14	103	115	107
<i>Ceratitis cosyra</i>	0.12	0.15	0.14	0.12	0.14	0.13	100	110	105
<i>Ceratitis rosa s.s.</i>	0.10 (0.14)*	0.13 (0.16)*	0.12	0.10 (0.15)*	0.13 (0.16)*	0.12	73	99	87

According to Steck and Ekesi (2015) measurements of the cephalopharyngeal skeleton can be used to show differences between species. The measurements in table 4 indicate that the mandibles of *B. dorsalis* have the longest distance between the ventral apodeme and the apical tooth (a) and between the dorsal apodeme and the ventral apodeme (b), with *C. rosa* s.s having the shortest distances of a and b (fig. 3). The measurements from the ventral apodeme and the apical tooth (a) and from the dorsal apodeme to the ventral apodeme (b) recorded in our study for *C. rosa* s.s differ from the corresponding measurements published by Steck & Ekesi (2015). Since the measurements we recorded from *C. capitata* were much the same as those recorded by Steck and Ekesi (2015), the difference between the measurements for *C. rosa* s.s. between the two studies could not be due to measurement error. Instead, the difference in measurements between the studies indicates a real difference in the size of the mandibles measured, as the larvae for both our study and Steck and Ekesi's (2015) study were obtained from the same colony. The difference in size might be due to artificial selection taking place in laboratory colonies (Steck and Ekesi 2015). Because of this difference in the size of the mandibles, only the ventral angle between the apical tooth and the ventral apodeme (c) in fig. 3 can be used as a reliable characteristic for distinction between the species. This angle was the most acute in *C. rosa* s.s, but did not differ between *B. dorsalis*, *C. capitata* and *C. cosyra*.

The validation of the morphological technique developed here for the identification of third instar fruit fly larvae showed that the percentage of positive identification of *C. rosa* s.s, *B. dorsalis* and *C. cosyra* was 100% whilst that of *C. capitata* was 93.75% (one misidentification over 16 tests by 3 users). The slide of *C. capitata* was wrongly identified as *B. dorsalis*.

2.4 Conclusion

Here we described a useful tool for a quick and initial differentiation between the third instar larvae of *C. capitata*, *C. rosa* s.s., *C. cosyra* and *B. dorsalis* detected in fruit using morphometrics. The geometric morphometric analysis of the mandibles using SHAPE analysis was found to be an effective tool for distinguishing between third instar larvae of *B. dorsalis* and *C. capitata*. This outline analysis approach is comparable to the use of landmarks to discriminate between closed shapes (Dujardin et al. 2014). In our study, third instar larvae of *C. rosa* s.s. and *C. cosyra* could not be distinctly separated using SHAPE analysis. The measurements of the distances between (1) the ventral apodeme and the apical tooth and (2) the dorsal apodeme and the ventral apodeme separated *C. rosa* from *C. cosyra*, but not *C. capitata* from *C. cosyra*. The more acute angle between the dorsal apodeme and the ventral apodeme of the mandibles of *C. rosa* s.s. can be used to further differentiate between *C. rosa* and *C. cosyra*.

The method described here is, however, limited to the third instar larvae of only four tephritid species which are pests of commercial fruit in South Africa and which are of phytosanitary concern for produce exported from of South Africa. This study nevertheless demonstrates the

potential for the development and use of geometric morphometric analysis for comparing a wider range of fruit fly species in a region, or even worldwide.

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Chapter 3

Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)?

Published as: PIETERSE, W., TERBLANCHE, J.S. & ADDISON, P. 2017. Do thermal tolerances and rapid thermal responses contribute to the invasion potential of *Bactrocera dorsalis* (Diptera: Tephritidae)? *Journal of Insect Physiology* **98**: 1-6

3.1 Introduction

The true fruit flies (Tephritidae) are destructive pests of many fruits and vegetables and are of quarantine importance for the export market (Ekesi *et al.* 2007). The invasive oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is of Asian origin and was first detected in Kenya in 2003 (Lux *et al.* 2003). It has showed a remarkably rapid invasion of the African continent (Drew *et al.* 2005) raising questions surrounding its environmental niche breadth (De Villiers *et al.* 2015) and possible climate change responses (Hill *et al.* 2016). First described as *B. invadens* when it arrived on the African continent in 2005 (Drew *et al.* 2005), it was subsequently synonymized with *B. dorsalis* (Hendel) (Schutze *et al.* 2015a) and re-described by Schutze *et al.* (2015b).

Currently *Bactrocera dorsalis* occurs in the northern and eastern parts of South Africa, with the Western Cape Province still free of *B. dorsalis* (Manrakhan *et al.* 2015) and therefore poses a significant risk to this large, productive deciduous fruit growing region. The presence of *B. dorsalis* in this province will have a serious impact on the export of fruit, as well as hamper fruit fly management strategies.

Extreme temperatures can be detrimental to insect (and other ectotherm) populations either through indirect effects such as activity constraints, or through direct effects including, for example temperature-induced sterility and cellular or tissue injury, resulting in functional limits or mortality (discussed in e.g. Sinclair *et al.* 2012, Andrew and Terblanche 2013). For insects, extreme temperatures can be a significant factor determining population dynamics (reviewed in Terblanche *et al.* 2011; 2015) but understanding when and where such constraints might exist requires foregoing knowledge of a species' thermal tolerances, with due consideration of life-stage-related variation (Bowler and Terblanche 2008). Given that insect populations may cope with thermal extremes using a diverse array of mechanisms and strategies (Andrew and Terblanche 2013), it is also critical that some understanding of phenotypic plasticity of these acute tolerances is obtained (Sgro *et al.* 2016).

The temperature tolerances for *B. dorsalis* adults and immature stages have been explored previously (e.g. Vargas *et al.* 1996; 1997, Ekesi *et al.* 2006, Chen *et al.* 2006, Rwomushana *et al.* 2008, Ye and Liu 2005). Survival, reproduction and longevity under different temperature regimes are important factors to consider when fruit flies are accidentally introduced into new areas and likely form a critical part of the invasion process (i.e. overcoming a key population establishment barrier). For example, extreme high temperatures (minimum temperature of 24°C and maximum temperature of 35°C) reduced the population growth of *C. capitata*, *B. dorsalis* and *B. cucurbitae* and was more limiting for establishment than lower temperatures (Vargas *et al.* 2000). The ability to rapidly alter tolerance and survival through phenotypic plasticity (physiological adjustments) may also improve the survival capacity of a species introduced to a novel environment, as illustrated for Tephritidae by Nyamukondiwa *et al.* (2010) with *C. capitata* relative to *C. rosa*. The ability to survive low temperatures was markedly improved after pre-treating *C. capitata* and *C. rosa* for 2 h at 5°C and 10°C. The survival of *C. capitata* increased after pre-treatment at 0°C and 35°C, but a full (100% survival) hardening response was not achieved (Nyamukondiwa *et al.* 2010). There was a marked difference in the speed and duration of the low temperature plastic responses of these two species which could manifest as an improvement in population survival upon exposure to fluctuating low temperatures in which the opportunities to harden occur intermittently. However, the importance of plastic hardening responses across a wider range of Tephritidae has generally been poorly examined (Nyamukondiwa *et al.* 2010). Hu *et al.* (2014) showed that *B. dorsalis* possess a heat hardening response at 35°C, 37°C, 39°C and 41°C; and has a wider distribution range as well as a higher thermal plasticity than *B. correcta*. However, *B. dorsalis*' thermal responses relative to other economically-important Tephritidae in South Africa has been poorly explored to date and is essential to understand the relative risks of establishment and population persistence if accidentally introduced in this growing region.

Various niche or distribution models (Stephens *et al.* 2007, Hill and Terblanche 2014, De Villiers *et al.* 2015) predict the spread of *B. dorsalis* to areas further south within South Africa. This region (specifically, the Western Cape Province of South Africa) has a warm, temperate climate, with cool, wet winters and hot, dry summers. According to De Meyer *et al.* (2010), *B. dorsalis* prefers hot and humid environments, but can survive dry seasons. *Bactrocera dorsalis* was observed in areas of West Africa, the Sudan and Zambia where the climate has longer dry periods and hot conditions prevail during part of the year (De Meyer *et al.* 2010). Physiological traits and genetic adaptation to new host plants might also influence the ability of *B. dorsalis* to establish in areas predicted to be suitable and thus alter its fundamental niche during invasion (Hill and Terblanche 2014). Climate change might not increase the possibility of *B. dorsalis* to establish in the Western Cape (Hill *et al.* 2016), but the adaptive capacity of traits of the flies'

thermal performance and survival will likely influence its ability to invade a new environment (Sinclair *et al.* 2012, Sgro *et al.* 2016).

The aims of this study were therefore to determine acute temperature survival rates for several key life-stages of *B. dorsalis*, and to determine whether *B. dorsalis* have acute plasticity (i.e. can rapidly cold- or heat-harden (RCH or RHH, respectively)) that might aid their survival and population establishment in this geographic region, which is generally considered less favorable (De Meyer *et al.* 2010, De Villiers *et al.* 2015). The colony of flies we used for our experiments came from the recently invaded sub-tropical Limpopo Province in South Africa and the experiments were conducted after 11 months in the laboratory. Limpopo Province was first invaded during 2008, and then declared fully established in 2015 (Manrakhan *et al.* 2015). Most other experiments on the thermal survival rates of *B. dorsalis* were conducted using flies that originated from the tropics (Vargas *et al.* 1996, 1997, Ekesi *et al.* 2006, Rwomushana *et al.* 2008) and being maintained for extended time periods in laboratory colonies. We were also interested in determining which life stage(s) are most resistant to thermal extremes to gain a better understanding of the species invasion ability to aid pest management or eradication efforts. To further understand the relative invasion risk we make use of comparisons with similar data presently available on con-familial species that possess both broader and narrower geographic distributions, but can be readily found in the region.

3.2 Materials and methods

3.2.1 Laboratory culture and rearing conditions

Bactrocera dorsalis was reared in the Insect Quarantine Facility of the Agricultural Research Council in Stellenbosch. The culture was started from infested guavas collected near Thohoyandou in Limpopo Province during March 2014 and wild flies from the same area were added once a year. They were reared at 27°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in PerspexTM cages (30 x 30 x 40 cm, 36 l) with a fabric sleeve under natural light conditions and provided with perforated apples for oviposition, as well as water and a mixture of sugar and yeast as food (Barnes *et al.* 2007). After removing the perforated apple halves from the adult cages, they were placed in plastic fast food bowls with artificial larval rearing medium (Barnes *et al.* 2007) with the addition of 100g carrot powder per kg of mix. The fast food bowls were placed in a plastic box with vermiculite in the bottom and incubated at 27°C ($\pm 1^\circ\text{C}$). Third instar larvae jumped from the bowls with rearing medium into the vermiculite on the bottom of the plastic box for pupation. Seven days after placing the apple on the rearing medium, the vermiculite was sifted daily to remove the pupae. Pupae were placed in honey jars marked with the day of collection and the flies that emerged were placed into new cages, marked with the day of emergence. The flies used in the experiments were 14 (± 1 day) days old. The age of eggs and

pupae used in the experiments were $12 \text{ h} \pm 1 \text{ h}$. Third instar larvae were used to gain insight into the larval stage.

3.2.2 Thermal plasticity

Upper and lower lethal temperatures (ULT and LLT, respectively) that cause 80 – 100% mortality using 2 h exposures (termed the ‘discriminating temperature’) were determined in preliminary trials and based on foregoing information for other Tephritidae (e.g. Nyamukondiwa *et al.* 2010 and Terblanche *et al.* 2010). For all hardening assays, six replicate 60 ml vials of 10 insects (adults) each were placed in a growth chamber at benign rearing conditions (27°C) for 30 min, after which flies were exposed to a range of acute temperatures in programmable incubators, growth chambers and liquid baths for 2 h before plunging vials containing flies directly into a refrigerated circulating liquid bath (filled with 95% EtOH for sub-zero operation) (Grant GP200-R2, Grant Instruments, UK) set at the discriminating temperature (-6.5°C or 42.7°C). Flies were returned to 27°C for 24 h before scoring survival. Survival was defined as a coordinated response to mild stimulation (e.g. prodding) or normal activities (e.g. mating, walking and flying). Six vials with ten flies in each were used as controls, not subjected to any treatment and kept at 27°C during the treatment and recovery time. They were handled in the same way as the treatment flies to control for handling effects that may induce heat shock proteins and could alter conclusions about mortality rates. All flies had access to food and water during the recovery time of the experiments. The cold hardening response of *B. dorsalis* was tested using exposure to 5°C, 10°C and 15°C while heat hardening was assessed using 30°C, 35°C, 37°C and 39°C as hardening temperatures. Gap treatments where flies were kept at 0°C and 5°C and 30°C, 35°C, 37°C and 39°C for two hours and then placed at 27°C for 1 h exposure to the discriminating temperatures at -6.5°C or 42.7°C were also included. Gap treatments for high and low temperature hardening responses were included to provide time for the development of proteins or cryoprotective sugars and polyhydric alcohols (e.g. proline, glycerol or sorbitol for low temperature responses (Misener *et al.* 2001) and various heat shock proteins that can take 20 minutes or longer to develop (Hoffmann *et al.* 2003, Sørensen and Loeschcke 2001), assuming that similar mechanisms underlie potential responses in *B. dorsalis* (Fig. 1).

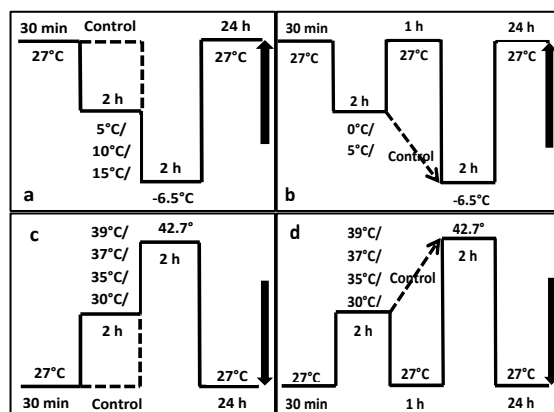


Figure 1. A graphical representation of the experimental treatments used to test the effect of (a) pre-treatment at 5°C, 10°C and 15°C for 2 h on the ability of *Bactrocera dorsalis* to survive the low discriminating temperature (-6.5°C for 2 h); (b) pre-treatment at 0°C and 5°C with a 1 h gap recovery time at 27°C before exposure to the discriminating temperature (-6.5°C for 2 h); (c) pre-treatment at 30°C, 35°C, 37°C or 39°C for 2 h on the ability of *Bactrocera dorsalis* to survive the high discriminating temperature (42.7°C for 2 h); and (d) pre-treatment at 30°C and 35°C with a 1 h gap recovery time (27°C for 1h) on the ability of *Bactrocera dorsalis* to survive the high discriminating temperature (42.7°C for 2 h). Arrows indicate the survival assessment timepoint.

3.2.3 Life stage comparison

Six replicate 60 ml vials of either 10 third instar larvae, 10 pupae, 10 adults or 50 eggs each were placed in a growth chamber at 27°C for 30 min, after which the vials containing the eggs, larvae and pupae were plunged directly into a refrigerated circulating liquid bath (filled with 95% EtOH for sub-zero operation) set at lethal (discriminating) temperatures (42.7°C for high temperature responses, -6.5°C for low temperature responses) for two hours. All life stages were returned to 27°C for 24 h before scoring survival.

3.2.4 Species comparison

For the species comparisons, the most comparable data for *C. capitata* and *C. rosa* were taken from Nyamukondiwa *et al.* (2010). The cold hardening response of *B. dorsalis* was tested using exposure to either 15°C, 10°C or 5°C, and heat hardening was tested at 35°C following plunge protocols described by Nyamukondiwa *et al.* (2010).

3.2.5 Statistical analyses

To determine the upper and lower discriminating temperatures (expecting c. 20% survival rate based on the pilot data from adult flies) a probit model (and its 95% confidence intervals) was fitted to the replicated survival data against temperature using SAS software (PROC PROBIT, v. 9.4 SAS Institute, Cary, NC, USA). The probit model assumes that survival is a binary probability (an individual is either 'alive' or 'dead'). To determine the effects of heat and cold hardening a Generalized Linear Model analysis corrected for overdispersion were fitted to the mean percentage survival data using a binomial distribution and the logit link function in SAS (v.

9.0, SAS Institute, Cary, NC, USA). Graphs of the effects were drawn using Statistica 7.0 (Statsoft, Tulsa, USA).

3.3 Results

3.3.1 Thermal plasticity

A brief (2 h) cold exposure at a mild (non-lethal) temperature resulted in significant increased survival compared to the control group at the two lower temperatures (5°C and 10°C) at the discriminating temperature of -6.5°C (DF 4, Wald's $\chi^2 = 37.24$, $p < 0.0001$) (Fig. 2a). Exposing *B. dorsalis* to 0°C and 5°C with a gap treatment of 1 h (Fig. 2b) did not improve subsequent survival rates when exposed to the discriminating temperature (-6.5°C). The same percentage of flies as in the control (5% $\pm 3.4\%$) survived the gap treatment at 0°C. At the 5°C gap treatment, 1.7% ($\pm 1.7\%$) flies survived compared to 3.3% ($\pm 2.1\%$) survival in the control group.

The survival of *B. dorsalis* was improved by heat hardening at the two higher temperatures (37°C and 39°C) compared to the control group (DF 5, Wald's $\chi^2 = 120.97$, $p < 0.0001$) (Fig. 2c) when exposed to the high lethal temperature of 42.7°C. Exposing *B. dorsalis* to 30°C and 35°C as hardening temperatures with a gap treatment of 1 h (Fig. 2d) did not improve the ability of the fly to survive the high lethal temperature (42.7°C). Only one fly survived in each treatment group and in the 30°C control group, with no survivors in the 35°C control group.

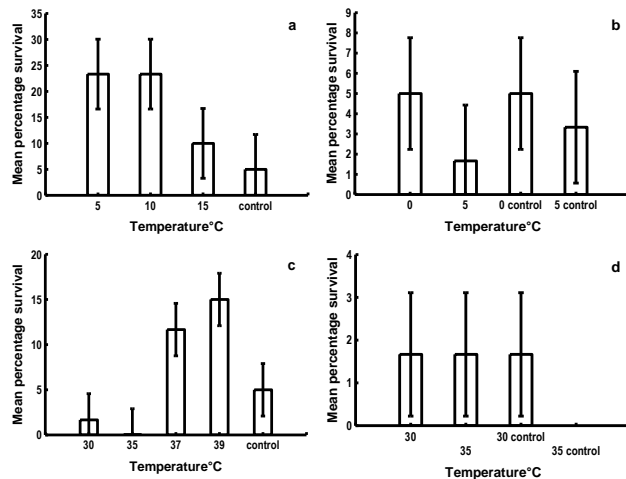


Figure 2. (a) The effect of pre-treatment at 5°C, 10°C and 15°C for 2 h on the ability of *Bactrocera dorsalis* to survive the low discriminating temperature (-6.5°C for 2 h). (b) Survival rates of *B. dorsalis* investigating the effect of pre-treatment at 0°C and 5°C with a 1 h gap recovery time at 27°C before exposure to the discriminating temperature (-6.5°C for 2 h). (c) Comparison of the effect of heat hardening at 30°C, 35°C, 37°C and 39°C for 2 h on the ability of *Bactrocera dorsalis* to survive the high discriminating temperature (42.7°C for 2 h). (d) The effect of heat hardening at 30°C and 35°C with a 1 h gap recovery time (27°C for 1h) on the ability of *Bactrocera dorsalis* to survive the high discriminating temperature (42.7°C for 2 h). Vertical bars denote 95% confidence intervals. Note that each panel's y-axis scale differs for clarity among groups.

3.3.2 Life stage comparison

There is a highly significant (DF 3, Wald's $\chi^2 = 491.62$, $p < 0.0001$) difference in the survival rates the life stages. No larvae or pupae survived the low discriminating temperature (-6.5°C) exposure for 2 h, but 60% ($\pm 4.2\%$) of the eggs and 5% ($\pm 3.4\%$) of the adults survived (Fig. 3). The eggs (44% $\pm 2.3\%$), pupae (56% $\pm 4.9\%$) and some adults (7% $\pm 6.6\%$) survived the high discriminating temperature (42.7°C), with no larval survivors. The eggs (60% $\pm 4.2\%$) and adults (5% $\pm 3.4\%$) were the only life stages able to survive the low discriminating temperature (-6.5°C). The third instar larval stage was the most vulnerable to extreme temperatures in this experiment. The pupae were the most heat resistant life stage and the eggs were able to withstand both the high and low discriminating temperatures.

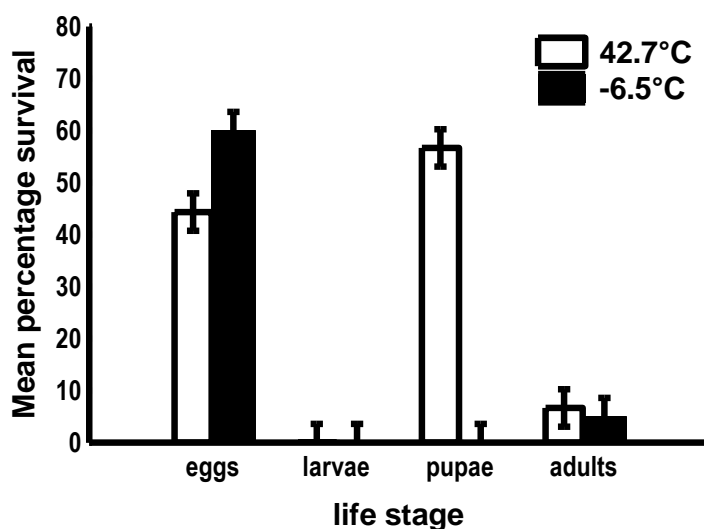


Figure 3. Ability of the different life stages (eggs, larvae, pupae and adults) of *Bactrocera dorsalis* to survive the high (42.7°C) and low (-6.5°C) discriminating temperatures for 2 h exposures. Vertical bars denote 95% confidence intervals.

3.3.3 Species comparison

There was a significant difference in the rapid cold hardening effect at the $p < 0.05$ level for the three temperatures and three species tested. The interaction between temperature and species was highly significant (DF 5, Wald's $\chi^2 = 796.12$, $p < 0.0001$) (Fig. 4a). Both *C. capitata* and *C. rosa* showed a significant cold hardening response after exposure to 5°C and 10°C for 2 h, but *B. dorsalis* did not.

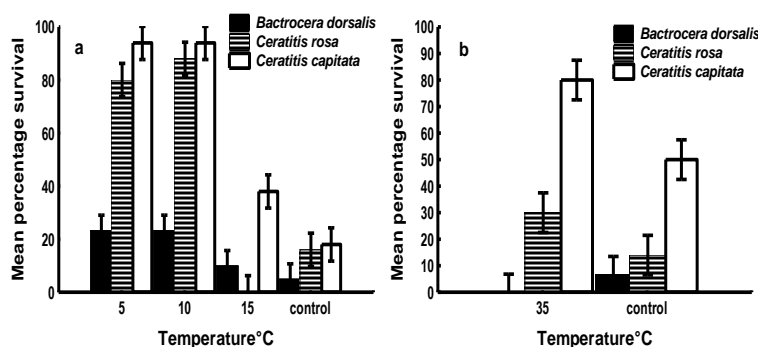


Figure 4. (a) Comparison of the effect of cold hardening at 5°C, 10°C and 15°C for 2 h on the ability of *Bactrocera dorsalis*, *Ceratitis capitata* and *Ceratitis rosa* to survive the low discriminating temperature (-6.5°C). (b) Comparison of the effect of heat hardening at 35°C for 2 h on the ability of *Bactrocera dorsalis*, *Ceratitis capitata* and *Ceratitis rosa* to survive the high discriminating temperature (42.7°C). Data used for the hardening responses of *C. capitata* and *C. rosa* are taken from Nyamukondiwa *et al.* (2010). Vertical bars denote 95% confidence intervals.

There was a statistically significant difference in the rapid heat hardening responses of the three species at the $p < 0.05$ level at 35°C (DF 2, Wald's $\chi^2 = 16.19$, $p < 0.001$) (Fig. 4b) when compared to the control at 35°C. *Ceratitis capitata* was able to heat harden significantly better than the control treatment while *C. rosa* and *B. dorsalis* were not able to develop a statistically significant heat hardening response within the conditions tested.

3.4 Discussion

The survival of alien or introduced species in a novel environment can be influenced by both basal stress resistance and also the phenotypic responses of the species to mild or extreme thermal conditions (Chown *et al.* 2007, Nyamukondiwa *et al.* 2010) or rapid adaptation of thermal traits (e.g. Foucaud *et al.* 2013, Gibert *et al.* 2016). The difference between temperature extremes are greater in a Mediterranean climate, such as the Western Cape of South Africa, than in the tropical climates that *B. dorsalis* originates from, which might create conditions that are more conducive to the survival of *B. dorsalis* if plasticity responses can expand the thermal range and respond over the time-course of the variability in a predictable way (Sgro *et al.* 2016). However, a test of this notion for *B. dorsalis* in the context of its invasive ability has not been previously undertaken.

The minimum temperature in the Western Cape fruit growing areas ranges between -2°C and 10°C (CapeFarmMapper v. 2.0.2.5), although it seldom drops below 0°C (see also climatological data in Nyamukondiwa *et al.* 2013). Here we found that *B. dorsalis* showed a small but statistically significant improvement in low temperature survival (-6.5°C for 2 h) after exposures to mild hardening temperatures of 5°C and 10°C for 2 h when compared to the control group. Only 23.3% ($\pm 5.58\%$) of flies survived after exposure to 5°C as a hardening treatment, with 23.3% ($\pm 10.54\%$) surviving after a hardening treatment at 10°C. These survival

rates do not greatly exceed the survival rate of $\pm 20\%$ expected for the discriminating temperature determined in a separate set of trials. If a population of *B. dorsalis* establishes in the Western Cape, it would therefore probably die out during the winter and be unable to rely on rapid plastic responses induced over diurnal time-scales. Small insects can raise their body temperature by several degrees above the ambient temperature by “basking” in the sun (Digby 1955, Parry 1951, Willmer and Unwin 1981), which could influence the ability of *B. dorsalis* adults to survive exposure to low temperatures for short times. Since *B. dorsalis* can fly as far as 50 km (Shi *et al.* 2005), re-infestation can also occur during the hotter months from adjacent areas where the winter temperature does not go lower than 10°C, thus perhaps sustaining an overwintering population. Nevertheless, a more extensive set of experiments determining plastic and basal responses of multiple thermal traits, and undertaken across longer seasonal time-scales, would be essential to support this conclusion, especially given that divergent plastic responses have been reported among insect populations for traits of low temperature stress resistance or recovery from chilling (e.g. Kleynhans *et al.* 2014, Fischer *et al.* 2010, Hoffmann *et al.* 2005).

Average summer temperatures in the Western Cape (CapeFarmMapper v. 2.0.2.5) seldom exceed 36°C, but maximum daily temperatures can be as high as 41°C in the fruit production areas at certain times of the year (and see discussion in Nyamukondiwa *et al.* 2013). We found that *B. dorsalis* adults were capable of generating a rapid high temperature survival improvement (i.e. heat-hardened at 37°C and 39°C for two hours), so extreme heat might not be immediately lethal to the adult population, but might increase pupal and larval mortality (Rwomushana *et al.* 2008) although further work would be necessary to fully test different life-stages and their respective ability to mount plastic responses to diverse environmental stress.

The third instar larvae of *C. capitata* were apparently more heat resistant than eggs (Gazit *et al.* 2004), but we found here that the eggs of *B. dorsalis* were more heat resistant than the larvae. Gazit *et al.* (2004) however, used shorter exposure times of between 0.17 min at 52°C and 20 min at 46°C. This suggests that stress responses and plasticity of tolerances might be sensitive to the precise methods used (Terblanche *et al.* 2011), and therefore that further work could expand the range of conditions and durations assayed to assess the generality of the responses we documented. According to Ye and Liu (2005), the larvae of *B. dorsalis* die at temperatures lower than 15°C. Female frugivorous Tephritidae deposit their eggs in a chamber below the skin of the fruit. The larvae emerge and feed on the fruit pulp tunneling deeper into the fruit (Blomefield *et al.* 2015). This protects the eggs and larvae against temperature extremes, thus improving their chances of survival. However, further work determining their respective microclimates, operative temperatures and relative behavioral reliance on avoiding extremes is necessary (Woods *et al.* 2015).

Ceratitis capitata adults displayed a significantly greater ability to heat harden after exposure to 35°C for two hours than *C. rosa*, which did not develop a statistically significant hardening response under the same conditions. By contrast, *B. dorsalis* did not develop any hardening response compared to the control at this temperature. *Bactrocera dorsalis* is regarded as an aggressive invader, able to out-compete *Ceratitis* species in tropical Africa (Duyck *et al.* 2006, Mwatawala *et al.* 2006, Ekesi *et al.* 2009). *B. dorsalis* only developed a heat-hardening response at higher temperatures (37°C and 39°C). From several lines of evidence presented in this study, the abilities of *C. rosa* and *C. capitata* to survive high temperature exposures better and develop a rapid heat hardening response across a wider range of conditions (e.g. at the lower temperature of 35°C) and to a greater degree, suggests that *B. dorsalis* might not be able to out-compete *C. rosa* and *C. capitata* in the Mediterranean climate of the Western Cape of South Africa based on the thermal niche requirements alone. These results have significant implications for understanding the relative invasion risk of *B. dorsalis* in the region, but also suggest that the thermal niche has likely not contributed significantly to their invasion success into Africa as they appear relatively sensitive to acute extreme temperatures. A wider range of traits and conditions should be tested to assess the generality of this conclusion.

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Chapter 4

The use of Geometric Morphometric Analysis to illustrate the shape change induced by different fruit hosts on the wing shape of *Bactrocera dorsalis* and *Ceratitis capitata* (Diptera: Tephritidae)

Published as: PIETERSE, W., BENÍTEZ, H.A. & ADDISON, P. 2017. The use of Geometric Morphometric Analysis to illustrate the shape change induced by different fruit hosts on the wing shape of *Bactrocera dorsalis* and *Ceratitis capitata* (Diptera: Tephritidae). *Zoologischer Anzeiger* **269**: 110–116

4.1 Introduction

The true fruit flies (Diptera: Tephritidae) are destructive pests of many fruits and vegetables and are of quarantine importance for the export market (Ekesi et al., 2007). In South Africa, the three indigenous fruit fly species which affect production and export of commercial fruit are *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* s.l. Karsch and *Ceratitis cosyra* (Walker) (Blomefield et al., 2015). A fourth species of economic importance in South Africa is the exotic pest of Asian origin, *Bactrocera dorsalis* (Hendel) (Grout & Moore, 2015). The invasive oriental fruit fly *B. dorsalis* was first detected in Kenya in 2003 (Lux et al., 2003). It has shown a remarkably rapid invasion on the African continent (Drew et al., 2005) raising questions surrounding its environmental niche breadth (De Villiers et al., 2015) and possible climate change responses (Hill et al., 2016). First described as *B. invadens* when it arrived on the African continent in 2005 (Drew et al., 2005), it was subsequently synonymized with *B. dorsalis* (Schutze et al., 2015b) and a re-description was provided by Schutze et al. (2015a).

Currently *B. dorsalis* occurs in the northern and eastern parts of South Africa, with the Western Cape Province still free of *B. dorsalis* (Manrakhan et al., 2015) and therefore poses a significant risk to this large, productive deciduous fruit growing region. The presence of *B. dorsalis* in this province will have a serious impact on the export of fruit, as well as hamper fruit fly management strategies. The Western Cape Province is the largest producer of deciduous fruit in South Africa, exporting 41% of the deciduous fruit grown in the province. South Africa earned about ZAR7 billion in export revenue from deciduous fruit exports in 2015 (Key Deciduous Fruit Statistics, 2015). It is therefore important to obtain baseline data which could indicate the suitability of deciduous fruit as a host for *B. dorsalis* as this species has mostly been documented from tropical and subtropical fruits only (Mwatawala et al., 2006). Changes in morphology as a result of nutrition can further help us to understand how fruit flies utilize various hosts, and therefore provide us with key management information.

Both *C. capitata* and *B. dorsalis* utilize a wide range of fruit crops as hosts. Host fruit has been shown to influence the size and life history parameters of *C. capitata* (Carey, 1984, Krainacker

et al., 1987). The shape, size and fitness of fruit flies are influenced by the quality of food available to the larvae as shown by Canato & Zucoloto (1998) in their experiments investigating the effects of carbohydrate ingestion by *C. capitata*. Larvae of *C. capitata* can select the part of the fruit that will provide them with the best nutrients for optimum performance (Fernandes-da-Silva & Zucoloto, 1993). The amount of protein and salt in the diet influences the development of larvae, as demonstrated by Lemos et al. (1992) when rearing *C. capitata* larvae on a high protein and high salt meat diet. Furthermore, wing shape and size are critical elements in the dispersal capacity of insects (DeVries et al. 2010).

Geometric morphometrics (Adams et al., 2013) compares the relative positions of landmarks between individuals or groups. It focuses on shape variation and is accomplished through the "Procrustes paradigm". Landmark based geometric morphometric analysis was used in various studies for taxonomic purposes. Schutze et al. (2012) found that while wing size data based on Canonical Variate Analysis (CVA) of fifteen landmarks on the wings failed to discriminate between morphologically similar taxa within the *B. dorsalis* species complex, CVA analysis of wing shape data did discriminate between the species with 93.27% accuracy. Adsavakulchai et al. (1998) also found that several species in the *Bactrocera dorsalis* complex could be identified with 89.6% accuracy when using discriminant and cluster analysis of wing shape. The reliability of shape variation rather than variation in size was also confirmed by Gilchrist & Crisafulli (2005) when distinguishing between wild and mass reared *Bactrocera tryoni* (Froggatt) as well as by Marsteller et al. (2009) when they compared the wing shape and size of six cryptic species of the tephritid genus *Blepharoneura* on the host plant *Gurania spinulosa* (Cucurbitaceae). They found that wing shape differed significantly among all six species. However, few studies have investigated the effect of nutrition on wing size and shape of fruit flies.

Because host fruit influenced the wing shape of other tephritid species such as *B. tryoni* (Gilchrist & Crisafulli, 2005), we wanted to investigate whether the fruit crop that the larvae were reared on would cause phenotypic changes in the shape of the wings of *C. capitata* and *B. dorsalis* and compare the extent of the changes. We aim to use landmark based geometric morphometrics, as described by Klingenberg (2011), to compare the wing shapes of *B. dorsalis* and *C. capitata* reared on various deciduous and citrus hosts. If shape changes do occur between flies that are reared on different hosts under standard laboratory conditions, this could form a basis from which to explore how these shape changes translate into optimal host utilization, and therefore refinement of management strategies against fruit flies.

4.2 Materials and Methods

4.2.1 Data collection

Adult specimens of *B. dorsalis* and *C. capitata* were reared from different fruit hosts, namely nectarine ("Arctic star" and "Mongreb" white flesh nectarines), plum ("Fortune", orange flesh

plums with purple skins), apple ("Golden Delicious") and pear ("Bon Chretien") were used as test fruit in all the experiments. *Bactrocera dorsalis* adults reared from citrus (navel oranges) were also included in this study, since orange is a preferred host for *B. dorsalis* (Mwatawala et al., 2006). The fruit was purchased the day before it was needed in a shop selling fruit grown under good agricultural practices and left at 25°C overnight before using in the experiments.

All experiments were conducted in a quarantine insectary at the Plant Quarantine Station of the Department of Agriculture, Fisheries and Forestry (DAFF) in Stellenbosch, South Africa. The conditions in the insectary were controlled at 26°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity with a 12h photoperiod (L12:D12). One hour dawn and dusk conditions were simulated by connecting 40W bulbs to a time switch.

The *B. dorsalis* used in the experiments were reared in the Insect Quarantine Facility of the Agricultural Research Council in Stellenbosch. The culture was started from infested guavas collected near Thohoyandou in Limpopo Province during March 2014 and wild flies from the same area were added once a year. They were reared at 27°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in PerspexTM cages (30 x 30 x 40 cm, 36 l) with a fabric sleeve under natural light conditions and provided with perforated apples for oviposition, as well as water and a mixture of sugar and yeast as food (Barnes et al. 2007).

The *C. capitata* used in the experiments were reared in the Insect Rearing Facility at Welgevallen experimental farm, Stellenbosch University. The culture was started from pupae received from the colony held at Citrus Research International (CRI) in Nelspruit, Mpumalanga Province, South Africa. The colonies of *C. capitata* had been maintained at CRI since 1999 and are regularly refreshed by addition of wild males every 2 years. They were reared at 25°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in PerspexTM cages (30 x 30 x 40 cm, 36 l) with a fabric sleeve under natural light conditions and provided with perforated apples for oviposition, as well as water and a mixture of sugar and yeast as food (Barnes et al. 2007).

After removing the perforated apple halves from the adult cages, they were placed in plastic fast food bowls with artificial larval rearing medium (Barnes *et al.* 2007) with the addition of 100g carrot powder per kg of mix. The fast food bowls were placed in a plastic box (30 x 30 x 20 cm, 18 l) with vermiculite in the bottom and incubated at 27°C ($\pm 1^\circ\text{C}$) for *B. dorsalis* and 25°C ($\pm 1^\circ\text{C}$) for *C. capitata*. Third instar larvae jumped from the bowls with rearing medium into the vermiculite on the bottom of the plastic box for pupation. Seven days after placing the apple on the rearing medium, the vermiculite was sifted daily to remove the pupae. Pupae were placed in honey jars marked with the day of collection and the flies that emerged were placed into new cages, marked with the day of emergence. The *B. dorsalis* used in the experiments were 14 (± 1 day) days old and the *C. capitata* were 7 (± 1 day) days old.

Five pairs of 14 (± 1) day old *B. dorsalis* or five pairs of 7 (± 1) day old *C. capitata* were placed in separate 19 x 15 x 16 cm (4.5 l) insect cages and provided with water and a mixture of sugar

and yeast as food. One weighed test fruit was placed in each cage for 24 hours. The number of sting marks on the fruit was counted before the fruit was placed on vermiculite in individual 2 liter plastic boxes with cloth in the lid for aeration, for pupation. The vermiculite was sifted daily after 7 days and the number of pupae was recorded. The pupae were placed in honey jars with aerated lids for the adults to emerge.

Adults emerging from the pupae were killed by placing them in the freezer at -18°C and were then preserved in 90% ethanol. Thereafter, the wings were removed and mounted in Euparal on glass slides.

4.2.2 Data analysis

Images of the left and right wings of males and females were captured using a Nikon Eclipse 80i compound microscope fitted with an Optica digital camera using Optica Vision Pro ver. 2.7 software. Images of 49 *C. capitata* wings (left and right) reared on apple; 17 reared on pear; 93 reared on nectarine and 52 reared on plum (211 in total) as well as 87 *B. dorsalis* wings reared on orange; 35 reared on apple; 49 reared on pear; 21 reared on nectarine and 25 reared on plum (217 in total) were captured. Photos were digitized with TPSUtil version 1.58 and TPSDig2 version 2.17 (Rohlf, 2013). Fourteen landmarks (Bookstein's shape coordinates, Adams et al. (2004)) of which 12 were the same as used by Schutze et al. (2012) were used on the wings to capture shape and size (Figure 1, Table 1). Repeatability tests on measurements were initially performed by capturing images of 200 wings twice and twice landmarking the images (400 images) in an error file.

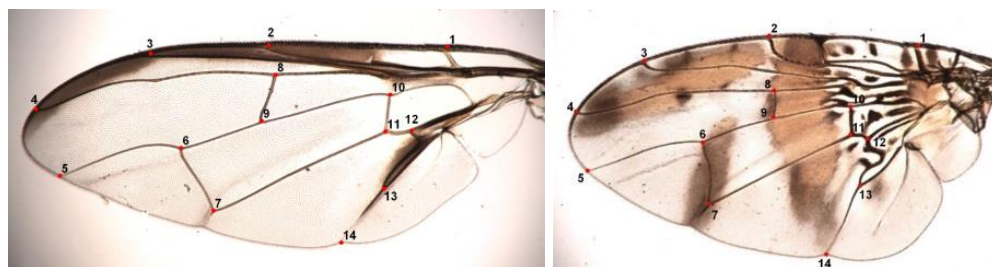


Fig. 1a & b. Landmarks on left wing of *Bactrocera dorsalis* (a) and *Ceratitis capitata* (b).

A Procrustes fit was applied to the landmark data using the program MorphoJ (Klingenberg, 2011). Procrustes fit is a process of scaling configurations to the same size, using centroid size; transposition to the same position of the center of gravity and rotation to the orientation that provides the minimum sum of squared distances between corresponding landmarks.

The imaging and digitizing error of this data were assessed using a Procrustes ANOVA to analyse the error file. The influence of sex and fruit kind on shape change of the wings was also assessed with a Procrustes ANOVA. Averaged values of all flies with the sexes combined were used for further analyses.

Table 1. Positions of the landmarks.

1	inner antero-distal corner of cell bc
2	junction of vein R1 and costal vein
3	termination of vein R2+3
4	termination of vein R4+5
5	termination of vein M
6	junction of vein M and dm-cu
7	junction of vein CuA1 and dm-cu
8	junction of vein R4+5 and r-m cross-vein
9	junction of vein M and r-m cross-vein
10	junction of vein M and dm-bm cross-vein
11	junction of vein CuA1 and dm-bm cross vein
12	junction of CuA1 and CuA2
13	junction of veins A1 and CuA2
14	termination of vein A1 and Cu2

To reduce the information to statistically unrelated factors, a principal component analysis (PCA) based on the covariance matrix of shape was performed on the 14 wing parameters. A covariance matrix pooled by sex was computed to compare if the effect of sexual dimorphism influences the results of the fruit kinds on wing shape. The first three principal components were analysed, since they contributed more than 50% of the shape change. Differences between fruit kinds were analysed using canonical variate analysis (CVA) and the results reported as the p-values of the Mahalanobis distances (a multivariate equivalent to the univariate difference scaled by the within-group standard deviation (Klingenberg & Gidaszewski (2010))). A multivariate regression analysis (MVRA) was calculated in order to determine the effect of size on shape (allometry) using the centroid size as an independent variable and the Procrustes coordinates as the dependent variable (Monteiro 1999).

4.3 Results

The mean square (MS) and F values of imaging error and digitizing error were much smaller than the Individual*Side interaction (Table 2). The Individual*Side interaction exceeds the Imaging error MS by six times and the Digitizing error by ten times. This indicates that the imaging of the wings and the digitizing of the landmarks were done accurately and the interaction of Individual*Side was due to the effect of fruit kind on the shape of the wings. Only the wing shape was used in all further analyses.

Table 2. Procrustes ANOVA results for shape change, showing imaging and digitizing error compared to the biological effect caused by the fruit hosts on wing shape.

Effect	SS	MS	df	F	P
Individual	0.25524189	0.0002798705	912	14.42	<.0001
Side	0.00634700	0.0002644583	24	13.63	<.0001
Individual*Side	0.01769948	0.0000194073	912	5.99	<.0001
Imaging error	0.00606129	0.0000032379	1872	1.78	<.0001
Digitizing error	0.00681719	0.0000018208	3744		

4.3.1 *Bactrocera dorsalis*

The effect of fruit kind on wing shape was found to be significant ($F = 11.99$, $p < 0.001$) (Table 3), while there was a further significant difference between male and female wing shape, indicating the presence of sexual shape dimorphism ($F = 85.77$, $p < 0.001$). The Fruit*Side interaction was not significant ($F = 0.93$, $p = 0.6677$) indicating that the fruit kind did not have an effect on the differences between the left and right wings of individual flies (Table 3).

Table 3. Procrustes ANOVA results for shape change, showing the effect of the fruit hosts and sex on the wing shape of *Bactrocera dorsalis*.

Effect	SS	MS	df	F	P	Pillai tr.	P (param.)
Fruit	0,0554125	0,000577214	96	11,99	<.0001	1,46	<.0001
Sex	0,0990934	0,00412889	24	85,77	<.0001	0,81	<.0001
Individual	0,1975577	4,81378E-05	4104	0,03	1	17,95	<.0001
Side	0,0072909	0,000303786	24	0,16	1	0,67	<.0001
Individual*side	6,8001166	0,001901599	3576	50,85	<.0001	16,35	1
Residual	0,0385907	3,73941E-05	1032				

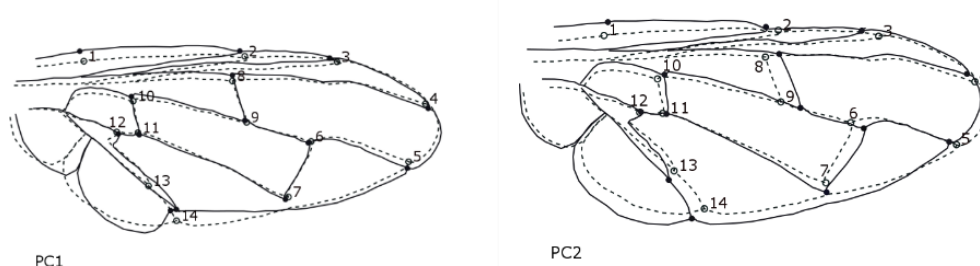


Fig. 2: Principle component (PC) shape outline showing 33.2% (PC1) and 20.7% (PC2) of the total variation that occurred in wing shape observed in *Bactrocera dorsalis* males and females during laboratory experiments. The dotted line and circle landmarks represent the average wing shape and the uninterrupted line and solid black landmarks represents the shift of the landmark associated with PC1 on a scale factor of 0.1.

The first three Principal Components (PCs) contributed 62.6% of the changes of the wing shape of *B. dorsalis*. After pooling the data the variance for the first three PCs decreased to 52.83%. Landmarks 1, 2, 3, 4, 5, 7 and 14 outline the wing (Fig. 2). PC1 indicates that a broadening of the wing occurred (landmarks 1, 2, 13 and 14), with landmark 5 indicating a slight elongation relative to the average shape of all wings. Landmark 13 showed the most change in shape by moving down and elongating cell *bcu*. PC2 indicates that a broadening of the wing occurred (landmarks 1, 2, 6, 7, 8, 9, 13 and 14), with landmark 5 indicating a slight shortening, relative to the average shape of all wings.

When analyzing the data using Canonical Variate Analysis (Fig. 3), the wings of flies reared on apple formed a clear separate grouping. The groups for citrus and pear overlapped as well as those for plum and nectarine.

The differences in Mahalanobis scores (the distance between the data point and centroid, see Brereton (2015) for a detailed explanation) between corresponding landmarks in the averaged wings of *B. dorsalis* flies were highly significant between individuals that were reared on nectarine, plum, apple and pear (Table 4), with the exception of the wings from plum and nectarine that differed significantly ($P = 0.0195$)

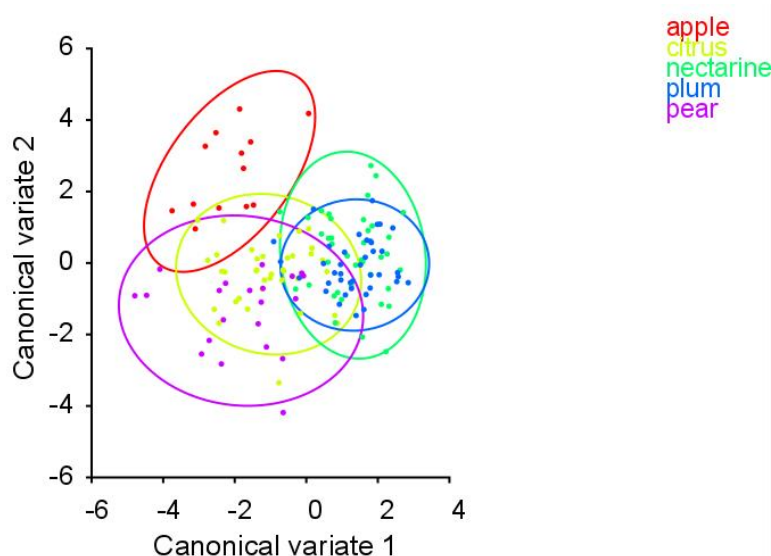


Fig. 3. Differences in the shape of *Bactrocera dorsalis* wings that were reared on different host fruits during laboratory experiments as indicated by the Canonical Variate Analysis. Citrus (ci), pear (pr) and apple (ap), nectarine (ne) and plum (pl). Confidence ellipses denote 95% probability.

Table 4. P-values from permutation tests (10000 permutation rounds) for Mahalanobis distances amongst fruit kinds for *Bactrocera dorsalis* and *Ceratitidis capitata*

		Apple	Citrus	Nectarine	Plum
Citrus	<i>B. dorsalis</i>	<0.0001			
	<i>C. capitata</i>	-			
Nectarine	<i>B. dorsalis</i>	<0.0001	<0.0001		
	<i>C. capitata</i>	<0.0001	-		
Plum	<i>B. dorsalis</i>	<0.0001	<0.0001	0.0195	
	<i>C. capitata</i>	<0.0001	-	<0.0001	
Pear	<i>B. dorsalis</i>	<0.0001	<0.0001	<0.0001	<0.0001
	<i>C. capitata</i>	0.0040	-	0.0003	0.0003

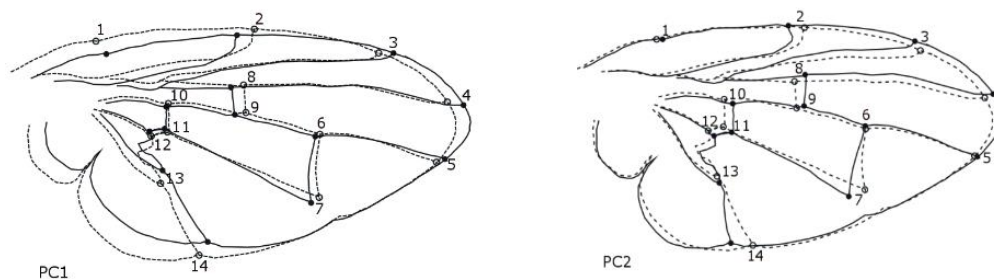


Fig. 4: Principle component (PC) shape outline showing 67.7% (PC1) and 5.19% (PC2) of the total that occurred in wing shape observed in *Ceratitidis capitata* males and females during laboratory experiments. The dotted line and circle landmarks represent the average wing shape and the uninterrupted line and solid black landmarks represents the shift of the landmark associated with PC1 on a scale factor of 0.1

The results of the multivariate regression analysis (Table 6) showed a weak relationship between the regression scores and centroid size. Size does not contribute significantly to shape change because the multivariate regression showed that only 7.8% of the change in wing shape was explained by size, which is not enough to make significant changes in the morphospace. Normally the percentage of allometry has to be bigger than 10% to make a clear or better visible contribution to the shape change.

4.3.2 *Ceratitidis capitata*

Significant differences in the shape of wings between males and females were found ($F = 172.19$, $p < 0.001$), indicating a clear wing shape sexual dimorphism (Table 5.). Significant differences were found for the wing shape of flies reared on different fruit kinds as extra effect ($F = 72$, $p < 0.0001$) (Table 5).

Table 5. Procrustes ANOVA results for shape change, showing the effect of the fruit hosts on wing shape of *Ceratitidis capitata*.

Effect	SS	MS	df	F	P	Pillai tr.	P (param.)
Fruit	0,0050475	7,01039E-05	72	2,01	<.0001	1,02	<.0001
Sex	0,1438349	0,005993121	24	172,19	<.0001	0,82	<.0001
Individual	0,1094258	3,48047E-05	3144	0,01	1	17,44	<.0001
Side	0,0017298	7,20756E-05	24	0,02	1	0,67	<.0001
Individual*side	9,0220693	0,003797167	2376	128,17	<.0001	15,17	0,9978
Residual	0,0355501	0,000029625	1200				

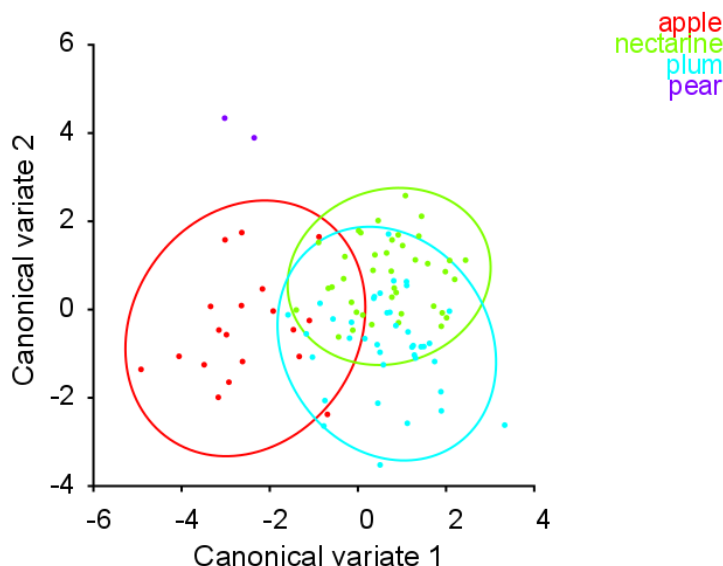


Fig. 5. Differences in the shape of *Ceratitis capitata* wings that were reared on different host fruits during laboratory experiments as indicated by the Canonical Variate Analysis. Pear (pr), apple (ap), nectarine (ne) and plum (pl). Confidence ellipses denote 95% probability.

The first three PCs contributed 77.95% of the changes of the wing shape of *C. capitata* in the covariance matrix without pooling by sex. After pooling the covariance matrix by sex, a considerable change appeared with the variance of the first three PCs decreasing to 58.2%. Landmarks 1, 2, 3, 4, 5, 7 and 14 outline the wing (Fig. 4). PC1 indicates that a narrowing of the widest part of the wing occurred (landmarks 13 and 14), with landmark 4 indicating a slight lengthening relative to the average shape of all wings. A broadening of the wing was observed in the second dimension of the shape space (PC2) with landmark 4 indicating a slight elongation of the distal part of the wing, relative to the average shape of all wings.

The values obtained from a Canonical Variate Analysis (Fig. 5), indicates that the wings of flies reared on apple form a clear separate grouping. The groups for nectarine and plum overlap.

Table 6. Results of the multivariate regression analysis analysing the relationship between the regression scores and centroid size.

	<i>Bactrocera dorsalis</i>	<i>Ceratitis capitata</i>
Total SS:	0.11883753	0.08521265
Predicted SS	0.00927917	0.00656926
Residual SS	0.10955836	0.07864339
% predicted	7.8083%	7.7093%
p-value	< 0.001	0.0014

The differences in Mahalanobis scores between corresponding landmarks in the averaged wings of *C. capitata* flies were highly significant between individuals that were reared on nectarine, plum and apple. (Table 4), with the exception of the wings from apple and pear ($P = 0.0040$), nectarine and pear ($P = 0.0003$) and plum and pear ($P = 0.0003$) that differed significantly

The results of the multivariate regression analysis (Table 6) showed a weak relationship between the wing shape and centroid size (7.7%, $p < 0.001$), indicating that this size influence is not significant in the morphospace of shape.

4.4 Discussion

The current study shows the importance of geometric morphometric analysis in studying shape adaptation correlated to nutritional factors. Our results indicate a significant wing shape variation for *B. dorsalis* and *C. capitata* reared from different host fruits under laboratory conditions.

According to Wootton (1981) the wing venation pattern of insect wings act as passive supports but also control the three-dimensional form of the wing. The first dimension of the shape space (PC1) of the PCA (simulation of morphospace in geometric morphometrics, Jolliffe (1986)) shows a noticeable variation of the wing in both *B. dorsalis* and *C. capitata* mediated by the vector movement of the landmark located at the Costal, Sub-costal and Radial veins with the result that the shape of the wings in *B. dorsalis* broadened while the wings of *C. capitata* narrowed. These adaptations to wing shape would change the wing kinetics which might influence the strength of the wing and beat patterns in flight (Wootton, 1981), and therefore possibly distribution potential.

Geometric morphometrics in flies has mostly been used for taxonomic purposes. One such study showed differences between species by comparing different wing morphotypes of the *B. dorsalis* complex (eg. Schutze et al. 2012), while Krosch et al. (2013) used a combination of molecular methods, measurements of the aedeagus and geometric morphometrics to identify the population structure of *B. dorsalis* in Malaysia. They concluded that integrating the methods provided finer definition of the populations than using only one method. Moraes et al. (2004) analyzed the divergence in wing morphology of the seven species in the *Drosophila buzzati* (Diptera: Drosophilidae) cluster and Boontop et al. (2017) used geometric morphometrics with molecular data (cytochrome oxidase subunit 1 (CO1) and microsatellites) to investigate the levels of variation in the populations of *Zeugodacus cucurbitae* (Coquillett) in Southeastern Asia and the West Pacific. In their study on population structure in *Ceratitis rosa* (Karsch) in which both molecular data and geometric morphometrics were utilized, Karsten et al. (2016) found no clear population differentiation and the presence of only one morphotype (R2) of the species at sites that had previously recorded two (R1 and R2) (Virgilio et al. 2013). The authors suggest that one reason for the absence of the R1 morphotype could be absence of a certain host plant at the time of sampling, suggesting developmental plasticity for these phenotypes (Karsten et al. 2013).

Geometric morphometrics has also been used to measure wing shape in response to environmental variables in insects. Benítez et al. (2013) used geometric morphometrics to analyse the hind wing shape of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae),

finding that the shape changed according to the major types of soil in Croatia. Our results showed that the centroid size did not change significantly (Table 5), but the shape of the wing varied considerably between fruit kinds. The insects reared on apple and pear showed more differences in wing shape compared to those raised on the other fruits, indicated by greater overlaps from confidence ellipses from CVA analyses (red and purple dots, Fig. 3 & Fig. 5). Navarro Campos et al. (2011) showed that wing size of *C. capitata* (measured according to traditional morphometric parameters) varied according to laboratory conditions and the different fruits used as host (apricot, peach, plum and orange).

Other studies using geometric morphometrics to evaluate shape changes induced by different fruit hosts found that host fruit influences the morphology of insects. Soto et al. (2010) found that genital and wing morphology of two sibling *Drosophila* species was strongly influenced by the different cactus hosts tested. Gómez-Cendra et al. (2016) reported that when they collected *Anastrepha fraterculus* (Wiedemann) from different geographic regions as well as from different host plant species, the flies differed morphologically according to host fruit, not geography. Nutritional stress and population density are some of the factors that can contribute to morphological changes in insects (Benítez, 2013, Benítez et al. 2015). Oroño et al. (2013) also detected genetic differences between adults of *A. fraterculus* reared from different host fruits (peach, walnut and guava) which grew in the same locality in Argentina. They hypothesize that the kairomones (a chemical substance emitted by one species that has an adaptive benefit, such as a stimulus for oviposition, to another species) in the fruit hosts utilized by feeding larvae might influence adult traits, such as the composition of pheromones. The phenotypic change in wing shape induced by host type might also influence mating success and male competitiveness (Gómez-Cendra et al., 2016). Sentinella et al. (2013) used the nutritional geometry approach to investigate the effects of protein and carbohydrate in the larval diet on growth and viability of *Telostylinus angusticollis* (Diptera: Neriidae) and found that body size increased with carbohydrate and, especially, protein content in the larval diet and egg-to-adult viability decreased with increasing protein content.

The species status of *B. invadens* in the *B. dorsalis* group was investigated by San José et al. (2013) using sequencing of the CO1, EF1 α (Elongation factor 1-alpha) and PER (PERIOD) genes. They concluded that none of the species forming part of the *B. dorsalis* clade are monophyletic and propose that the major pest species represent a single, phenotypically plastic species. The changes in wing shape might be a function of the plasticity of the species. It is clear that geometric morphometrics is an important tool to add to the methods used to study insects. This study is the first to use this method to study the influence of host fruit on the wing shape of *C. capitata* and *B. dorsalis*. The effect that these changes in wing shape induced by the different fruit hosts have on fruit fly fitness are as yet unclear and should be investigated further.

4.5 References

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Chapter 5

Comparative demography of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit

5.1 Introduction

Bactrocera dorsalis (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) are important pests of commercial fruit (White & Elson-Harris, 1992). They are both multivoltine species and do not enter a diapause phase (Burk & Calkins, 1983, Chen *et al.*, 2006, Goergen *et al.*, 2011). *Bactrocera dorsalis* is of Asian origin and was first recorded in Taiwan in 1912 (Shi *et al.*, 2005). *Ceratitis capitata* is of Afrotropical origin (De Meyer *et al.*, 2002) and was recorded as the most widespread fruit fly pest species (De Villiers *et al.* 2013) as well as the dominant species on deciduous fruit in the Western Cape Province (Manrakhan & Addison 2013, Blomefield *et al.*, 2015). *Bactrocera dorsalis* was first recorded in Africa from Kenya (Lux *et al.*, 2003) and was first declared established in Vhembe District, Limpopo Province in the northern areas of South Africa in 2013 (Manrakhan *et al.*, 2015). It is currently present in the northern and north-eastern parts of the country (Manrakhan *et al.*, 2015), but is still absent in other areas of the country. A number of regions in South Africa, including regions in the currently *B. dorsalis* free Western Cape Province, were, however, deemed suitable for establishment of *B. dorsalis* based on a recent climatic model (De Villiers *et al.* 2016). The Western Cape Province of South Africa is an important deciduous fruit growing region in the country (Anonymous, 2016). Most of the deciduous fruit being grown commercially are exported and brings important revenues to the country and the region (Anonymous, 2016). Fruit flies are pests of phytosanitary concern for export fruit markets from South Africa (Barnes *et al.*, 2015). As such, exclusion of pests and effective management of local pests are important strategies in fruit industries in South Africa to enable export of fruit fly free fruit (Barnes *et al.*, 2015).

Ceratitis capitata and *B. dorsalis* both exhibit a high reproductive potential, are highly mobile and are opportunistic, broad range exploiters of fruit (Chen *et al.*, 2006; Ekesi *et al.*, 2007). Host plants also play an important role in the ability of fruit fly species to survive and disperse (Malacrida *et al.*, 2007, Bateman, 1972). In Africa, *B. dorsalis* has been recorded on more than 80 host plants (De Meyer *et al.* 2012). Mango appears to be its primary host in many African countries (Mwatawala *et al.* 2004, Ekesi *et al.* 2006), with guava (*Psidium guajava*; Myrtaceae) (Vargas *et al.* 2007, Ali *et al.* 2014, Hussain *et al.* 2015) and tropical almond (*Terminalia catappa*; Combretaceae) also suitable reservoir hosts for the pest (Mwatawala *et al.* 2006, 2009). For *C. capitata*, 353 plant species were listed as hosts (Liquido *et al.*, 1990; Radonjić *et al.*, 2013). In the northern parts of South Africa, where *B. dorsalis* has been present since 2013, a limited host range was recorded for the pest (Theron *et al.* 2017). In another recent

survey on various indigenous fruits in the northern areas of South Africa, Grové *et al.* (2017) found that *B. dorsalis* only emerged from one indigenous fruit - marula fruit (*Sclerocarya birrea* (A.Rich.) Hochst. (Anacardiaceae)) out of a range of 28 plant species sampled. In that survey (Grove *et al.* 2017), *C. capitata* emerged from 12 of 28 indigenous plant species tested.

Literature on the utilisation of deciduous fruit by *B. dorsalis* is scarce. White & Elson-Harris (1992) listed *Prunus persica* (L.) Batsch, (Nectarine), *Prunus domestica* L., (Plum), *Malus domestica* Borkh. (Apple) and *Pyrus communis* L., (Pear) as host plants for *B. dorsalis* in China from various sources, some unpublished. Ye & Liu (2005) found that apple was a less preferred host for *B. dorsalis* in China. Pear was not infested as frequently as peach and could not support high numbers of *B. dorsalis* (Ye & Liu, 2005). It would be important to have an understanding of how effectively *B. dorsalis* would utilise deciduous fruits as hosts, as this could be a good predictor for establishment potential.

The main objectives of this study were therefore to compare the development, reproduction and survival of *B. dorsalis* and *C. capitata* on main deciduous fruit types cultivated in the Western Cape. With this information, the suitability of these deciduous fruit types to *B. dorsalis*, relative to *C. capitata* can be determined. Together with physiological temperature parameters (see Chapter three), a better estimation can be made of potential establishment potential of *B. dorsalis* in the currently pest free Western Cape Province.

5.2 Materials and Methods

5.2.1 Insect colonies

Bactrocera dorsalis was reared in the Insect Quarantine Facility of the Agricultural Research Council in Stellenbosch. They were reared at 27°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in PerspexTM cages (30 x 30 x 40 cm, 36 l) with a fabric sleeve under natural light conditions and provided with perforated apple halves for oviposition as well as water and a mixture of sugar and yeast as food (Barnes *et al.*, 2007). The culture was started from infested guavas collected near Thohoyandou in Limpopo Province during March 2014 and wild flies from the same area were added once a year. The perforated apple halves provided for oviposition were removed every two days. Larvae were reared on an artificial larval rearing medium (Barnes *et al.* 2007) with the addition of 100g carrot powder per kg of mix and kept in separate containers on vermiculite at 27°C ($\pm 1^\circ\text{C}$) for pupation. The vermiculite was sifted to remove the pupae, which were placed in honey jars marked with the date collected. The flies that emerged were released into cages marked with the day of emergence. The flies used in the experiments were 14 (± 1) days old. *Bactrocera dorsalis* reared in a colony under optimal conditions reaches sexual maturity between 10-15 days after emergence (Bess & Haramoto, 1961; Diatta *et al.*, 2013).

Ceratitis capitata was reared in the insect rearing facility at Welgevallen experimental farm, Stellenbosch University campus. They were reared at 25°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in PerspexTM cages (800 mm³) under 12h light/12h dark conditions and provided with perforated apple halves for oviposition as well as water and a mixture of sugar and yeast as food (Barnes *et al.*, 2007). Pupae to start the colony were obtained from colonies held at Citrus Research International (CRI) in Nelspruit, Mpumalanga province, South Africa. The perforated apple halves provided for oviposition were removed every two days. Larvae were reared on an artificial larval rearing medium (Barnes *et al.*, 2007) and kept in separate containers on vermiculite at 25°C ($\pm 1^\circ\text{C}$) for pupation. The vermiculite was sifted to remove the pupae, which were placed in honey jars marked with the date collected. The flies that emerged were released into cages marked with the day of emergence. The flies used in the experiments were 7 (± 1) days old. *Ceratitis capitata* reared in a colony under optimal conditions reaches sexual maturity between 4-6 days after emergence (Carey, 1884).

All experiments were conducted in a quarantine insectary at the Plant Quarantine Station of the Department of Agriculture, Forestry and Fisheries in Stellenbosch, South Africa. The conditions in the insectary were controlled at 26°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity with a 12h light/12h dark cycle. One hour dawn and dusk was created by connecting 40W bulbs to a time switch. A 40W bulb was switched on simulating 1 h dawn and 1 h dusk every day within the 12 h light cycle.

5.2.2 Deciduous fruit tested

Four deciduous fruit types were used in all tests: *Prunus persica* (L.) Batsch, Nectarine “Arctic Star” and Nectarine “Mongreb”; *Prunus domestica* L., Plum “Fortune”; *Malus domestica* Borkh., Apple “Golden delicious”; *Pyrus communis* L., Pear “Packham”. Tests were carried out between December 2016 and June 2017.

Fruit was purchased the day before it was needed in a shop selling fruit grown under good agricultural practice and left at 25°C overnight before using in the experiments.

5.2.3 Duration and viability of egg stage

For each fruit fly species tested, five adult pairs (female and male) were placed in 19 x 15 x 16 cm 4.5 l aerated insect cages and provided with water and a mixture of sugar and yeast as food (in a 3:1 ratio). One test fruit was placed in each cage for 24 hours. The test fruit was weighed before placement in the cage. The number of sting marks on the fruit was counted as well as the number of eggs per sting mark. All the eggs were dissected out and placed on moist black filter paper (9 cm in diameter, Macherey-Nagel GmbH & Co. KG) in sterile petri dishes. The petri dishes were kept at 25°C. Eggs were counted every hour for 8 hours until all eggs had hatched or no further egg hatch occurred. The number of larvae that hatched was recorded. The experiment was repeated four times using mixed cohorts with each fruit type.

5.2.4 Development and survival of immature stages

For each fruit fly species tested, five adult pairs (female and male) were placed in 19 x 15 x 16 cm (4.5 l) aerated insect cages and provided with water and a mixture of sugar and yeast (in a 3:1 ratio) as food. One test fruit was placed in each cage for 24 hours. The test fruit was weighed before the start of the test. The numbers of sting marks on the fruit were counted before the fruit was placed on vermiculite in individual 2 litre plastic boxes with cloth in the lid for aeration, for pupation. The vermiculite was sifted daily after 7 days and the numbers of pupae were recorded daily. The pupae were placed in honey jars with aerated lids for the adults to emerge. Adult emergence was recorded daily, noting the number of males and females emerging every day. The experiment was repeated four times using mixed cohorts with each fruit type.

5.2.5 Adult demographic parameters

For each fruit fly species tested, a pair of adult flies (female and male) were placed separately in 11 x 12 x 18 cm (2 l) aerated insect cages and provided with water and a mixture of sugar and yeast (in a 3:1 ratio) as food. Founder flies for this experiment were reared from fruit used in the previous experiment following the development and survival of immature stages of the two fruit fly species. The founder flies were then provided the same fruit type for oviposition instead of the perforated apple halves. Eggs laid in the specific fruit type were then left to develop in the fruit instead of being transferred to the artificial medium and reared for two generations. A 5 ml container (15 mm in diameter) with a 1 cm piece of test fruit covered with parafilm™ (pierced four times) was placed in each cage. The container with fruit was replaced daily and the number of sting marks, number of eggs and mortality of the adults were recorded daily for 90 days.

5.2.6 Statistical analysis

The following parameters were analysed: number of sting marks, number of eggs, percentage egg hatch, hours until egg hatch, number of pupae, number of pupae per gram of fruit, number of days to pupation, percentage adult emergence and number of emerged adults. Only mean values without standard deviation values are reported. The main effects and interactions between effects of species, fruit and time on the above mentioned parameters were analysed using factorial ANOVA analyses. Data collected over time for the number of eggs and sting marks produced over 90 days was amalgamated into 10 day periods for clarity of the graphs and analysed using Repeated Measures ANOVA. The rate of death was analysed using the Life Tables function of Statistica, calculating the cumulative proportion of flies surviving with the resultant Z value indicating the number of standard deviations below or above the population mean. Mean values were compared using least significant differences (LSD) post-hoc tests. All analyses were conducted using Statistica 7.0 (Statsoft, Tulsa, USA).

5.2.7 Life table parameters

Life table parameters of *B. dorsalis* and *C. capitata* on each fruit tested were determined based on data collected over 90 days in experiment 3 (adult demographic parameters).

The net reproduction rate (R_0) was determined using the following equation (Carey, 1982):

$$\sum_{x=1}^t l_x m_x$$

Where l_x is the proportion of females alive on day x , and m_x is the total number of female progeny produced per female on day x , were determined.

The mean generation time (T) was calculated using the following equation (Birch, 1948)

$$T = \frac{\sum x l_x m_x}{\sum l_x m_x}$$

where T is given as time in days.

These values were subsequently used to obtain an initial estimate of the intrinsic rate of natural increase (r_m) as described in Walton & Pringle (2005).

5.3 Results

5.3.1 Oviposition

Over 24 hours, *B. dorsalis* produced more eggs than *C. capitata* per female on all deciduous crops (Table 1). Similarly when exposed to fruit section over 90 days, *B. dorsalis* produced more eggs than *C. capitata* on all crops (Table 1 & Fig. 1). There was, generally (both species and both time periods: 24 H [$F_{(6, 46)}=9.5421$, $p<0.001$] and 90 days [$F_{(3, 7192)}=27.170$, $p<0.05$], a significant interaction (Table 2) between crop and number of eggs. Over 24 H, *B. dorsalis* deposited the lowest number of eggs on nectarine. Over 24 H, *C. capitata* deposited the lowest number of eggs on pear and the highest number of eggs on apple (Table 1). Over 90 days, *B. dorsalis* deposited the highest number of eggs on pear (Table 1 and Fig. 1) whilst *C. capitata* deposited the highest number of eggs on plum over the same time period. *Ceratitis capitata* deposited the lowest number of eggs on apple over 90 days. There was, generally (both species and both time periods: 24 H [$F_{(6, 46)}=9.5421$, $p<0.001$] and 90 days [$F_{(3, 7192)}=66.411$, $p<0.001$], a significant interaction between crop and number of sting marks. *Bactrocera dorsalis* produced the highest number of sting marks (indicating oviposition attempts) on nectarine ($p<0.05$), the only crop where it produced more sting marks than *C. capitata*. *Ceratitis capitata* females produced the lowest number and *B. dorsalis* the highest number of sting marks on apple. Both species produced about the same number of sting marks on plum over 90 days. There was no significant interaction between day*species*fruit when analysing the mean number of sting marks [$F_{(27, 720)}=0.74495$, $p=0.82310$], but there was a significant interaction between day*species*fruit when analysing the mean number of eggs deposited by a single

female on the four fruit types over 90 days [$F_{(27, 720)}=2.2090$, $p=0.00044$] (Fig 1.). *Bactrocera dorsalis* produced significantly more eggs on pear than on any other crop and also produced more eggs on nectarine and apple than *C. capitata* ($p<0.05$). *Bactrocera dorsalis* produced eggs over the full 90 day period on apple, the only crop where this occurred (Table 1).

Table 1. Mean numbers (\pm std dev) of eggs per female, mean number of sting marks per female and mean percentage egg hatch of *Ceratitis capitata* and *Bactrocera dorsalis* on four different deciduous fruit types. In each section, means followed by the same letters within the same row are not significantly different at 0.05% probability level.

Parameters tested	Fruit fly species	nectarine	plum	pear	apple
Mean numbers of:					
eggs per female (24 h)	<i>Bactrocera dorsalis</i>	40.3 ^a \pm 5.3	53.25 ^c \pm 17	54.5 ^c \pm 4.5	44.35 ^c \pm 13.5
	<i>Ceratitis capitata</i>	25.75 ^b \pm 3.5	18.5 ^b \pm 2.7	8.9 ^c \pm 4.2	38.5 ^c \pm 5.5
sting marks per female (24 h)	<i>Bactrocera dorsalis</i>	7.8 ^a \pm 2	1.05 ^c \pm 0.4	0.95 ^c \pm 0.4	0.9 ^c \pm 0.3
	<i>Ceratitis capitata</i>	3.7 ^b \pm 0.7	3.95 ^b \pm 2.6	1.3 ^c \pm 1	1.25 ^c \pm 0.3
eggs per female (90 days)	<i>Bactrocera dorsalis</i>	6.9 ^b \pm 17.2	7.7 ^b \pm 20.1	15.2 ^a \pm 28.8	7.4 ^b \pm 12.6
	<i>Ceratitis capitata</i>	4.2 ^c \pm 14.3	6.8 ^b \pm 15.5	4.8 ^c \pm 15.5	0.7 ^d \pm 3.7
sting marks per female (90 days)	<i>Bactrocera dorsalis</i>	0.60 ^c \pm 1.2	0.56 ^c \pm 1.2	0.90 ^b \pm 1.4	1.17 ^a \pm 1.4
	<i>Ceratitis capitata</i>	0.34 ^d \pm 1.3	0.57 ^c \pm 1.2	0.29 ^d \pm 0.8	0.13 ^e \pm 0.5
Mean percentage of:					
egg hatch for fruit (24 h)	<i>Bactrocera dorsalis</i>	88% ^{abc} \pm 5.9	81% ^{bc} \pm 11.3	73% ^c \pm 6.5	47% ^d \pm 23.2
	<i>Ceratitis capitata</i>	97% ^a \pm 1	91% ^a \pm 3.5	89% ^{abc} \pm 13.3	94% ^{ab} \pm 2.1

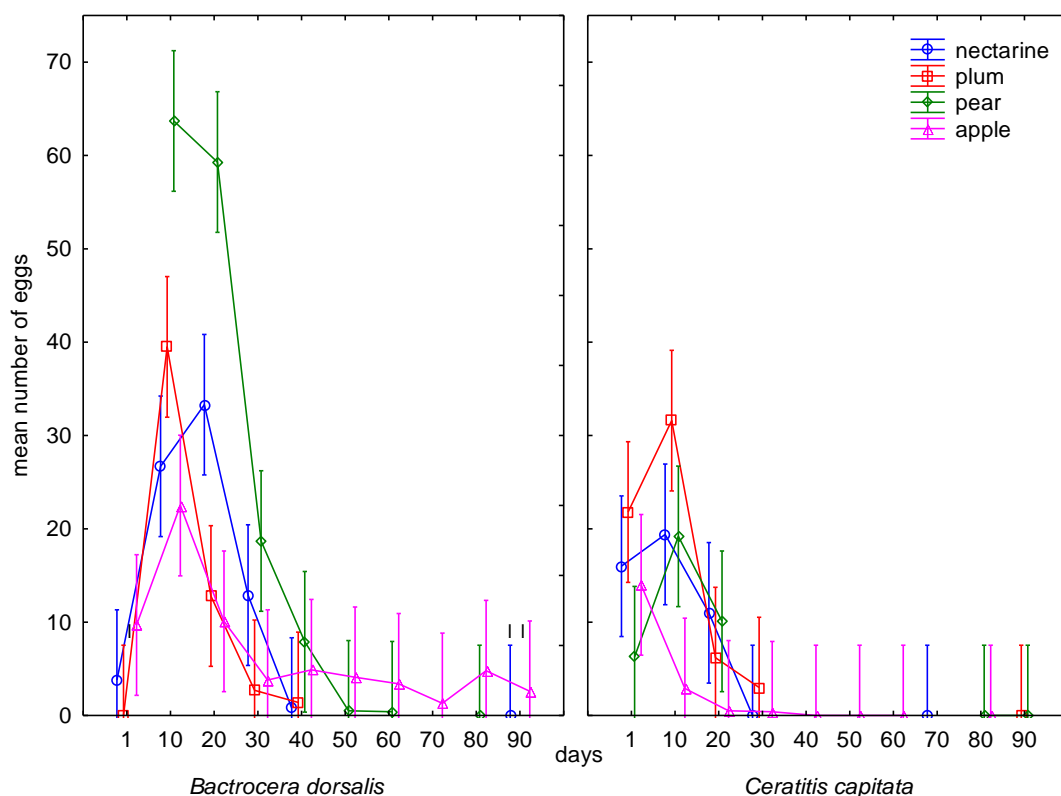


Figure 1. The mean number of eggs deposited daily per female over 90 days recorded for two fruit fly species (*Bactrocera dorsalis* and *Ceratitis capitata*) on four different deciduous fruit types. Vertical bars denote ± 0.95 confidence intervals. Values indicated by the same letter do not differ significantly at $p = 0.05$.

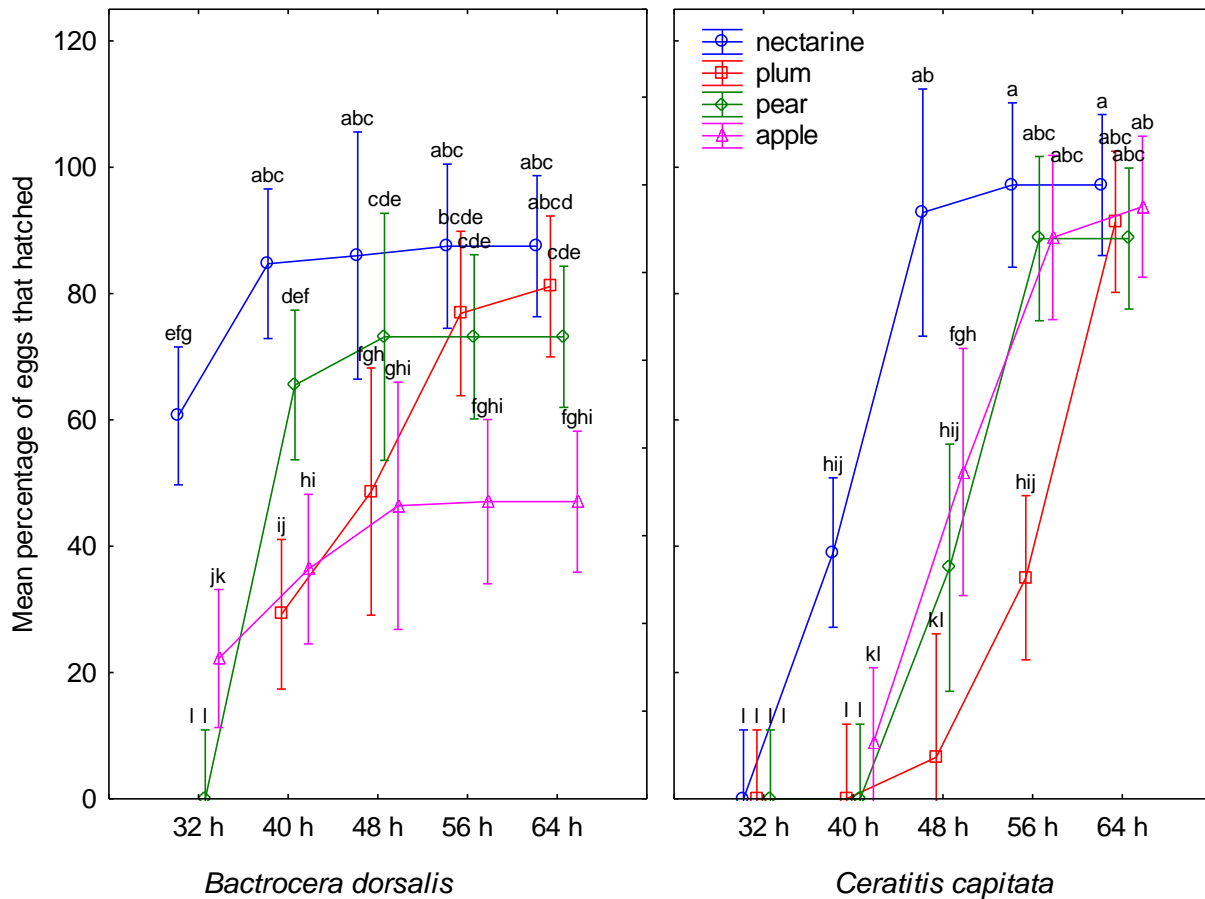


Figure 2. The mean number of eggs hatched recorded for two species of fruit fly on four different deciduous fruit types. \pm Vertical bars denote 0.95 confidence intervals. Values indicated by the same letter do not differ significantly at $p = 0.05$.

There was a significant interaction between time*species*crop when analysing the mean number of eggs that hatched [$F_{(12, 96)}=13.822$, $p<0.001$] (Fig 2). The eggs of *B. dorsalis* hatched sooner (after 32 hours) than those of *C. capitata* (after 40 hours). Eggs of both species hatched the fastest on nectarine and apple and the slowest on plum and pear.

For *B. dorsalis*, egg hatch was the lowest on apple and the highest on nectarine (Table 1). Averaged over all fruit types, the percentage egg hatch was significantly higher for the eggs of *C. capitata* than those of *B. dorsalis*.

Table 2. Mean number of pupae, mean percentage eclosion and the mean number of adults (for 5 *Ceratitis capitata* and *Bactrocera dorsalis* females over 24 hours) produced on four different deciduous fruit types. In each section and column, means followed by the same letters are not significantly different at 0.05% probability level. The main effect means are indicated in bold.

treatment	Mean number of pupae in 24 hours					Mean number of pupae per gram of fruit					Mean % pupal eclosion					Mean number adults				
	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
<i>Ceratitis capitata</i>	168 ^a	21 ^c	66 ^{bc}	55 ^{bc}	77.3^a	1.66 ^a	0.37 ^{cd}	0.37 ^{cd}	0.4 ^{cd}	0.7^d	95 ^a	92 ^a	94 ^a	84 ^b	91^a	162 ^a	19 ^c	63 ^{bc}	47 ^{bc}	73^d
<i>Bactrocera dorsalis</i>	103 ^b	47 ^{bc}	46 ^{bc}	27 ^c	55.4^d	1.1 ^{ab}	0.9 ^{bc}	0.3 ^{cd}	0.2 ^d	0.6^a	95 ^a	95 ^a	91 ^{ab}	95 ^a	94^a	98 ^b	45 ^{bc}	43 ^{bc}	25 ^c	53^a
means	136^a	34^b	56^b	41^b		1.4^a	0.7^b	0.3^b	0.3^b		95^a	94^{ab}	93^{ab}	90^b		130^a	32^b	53^b	36^b	

Table 3. Mean number of days to pupation and the mean number of days to adult emergence (for 5 *Ceratitis capitata* and *Bactrocera dorsalis* females over 24 hours) produced on four different deciduous fruit types. In each section and column, means followed by the same letters are not significantly different at 0.05% probability level. The main effect means are indicated in bold.

treatment	Mean no. of days to pupation					Mean no. of days to adult emergence				
	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
<i>C. capitata</i>	9 ^{de}	12 ^c	12 ^c	23 ^a	14^a	18 ^d	21 ^c	20 ^c	31 ^a	22.5^a
<i>B. dorsalis</i>	8 ^e	11 ^{cd}	12 ^c	19 ^b	12.5^b	18 ^d	20 ^c	22 ^c	27 ^b	21.8^a
means	8.6^c	11.6^b	12^b	21^a		18^c	20.5^b	21^b	29.4^a	

Table 4. The influence of nectarine, plum, pear and apple on developmental parameters of *Bactrocera dorsalis* and *Ceratitis capitata*.

	Nectarine				plum				pear				apple			
	min	max	mean +/- std dev		min	max	mean +/- std dev		min	max	mean +/- std dev		min	max	mean +/- std dev	
Number of adults <i>B. dorsalis</i>	50	153	97.8%+/- 43.4%		22	77	45 +/- 23.2		19	67	42.5 +/- 24		19	29	25 +/- 4.3	
Number of adults <i>C. capitata</i>	47	245	162.25 +/- 83.03		10	33	18.75 +/- 9.8		35	111	62.5 +/- 33.8		18	84	46.5 +/- 31.5	
% male <i>B. dorsalis</i>	28%	60.4%	48.27% +/- 14.03%		42.9%	62.2%	50.6% +/- 8.6%		40%	50.73%	48.5% +/- 6.3%		36%	59.25%	43.8% +/- 10.6%	
% male <i>C. capitata</i>	48.6%	57.7%	53.2% +/- 5.61%		30%	66.7%	50.7% +/- 15.2%		46.66%	51.42%	49.4% +/- 2.17%		26.08%	58.33%	46.1% +/- 14.7%	
Male lifespan (days) <i>B. dorsalis</i>	13	89	38.1 +/- 24.5		14	89	41.4 +/- 25.9		10	89	33.5 +/- 23.1		6	89	42.3 +/- 25.3	
Male lifespan (days) <i>C. capitata</i>	7	89	27.4 +/- 20.6		10	89	35.5 +/- 23.5		41	88	28.6 +/- 18.8		6	65	17.6 +/- 16.2	
% female <i>B. dorsalis</i>	39.6%	72%	51.7% +/- 14.03%		37.8%	57.1%	49.3 +/- 8.6%		46.21%	60%	51.5% +/- 6.3%		40.74%	64.%	56.2% +/- 10.6%	
% female <i>C. capitata</i>	42.3%	51.4%	46.8% +/- 5.2%		33.33%	70%	49.3% +/- 15.2%		48.57%	53.33%	50.6% +/- 2.2%		41.66%	73.91%	53.8% +/- 14.7%	
Female lifespan (days) <i>B. dorsalis</i>	11	71	25 +/- 17.02		12	89	29.1 +/- 21.9		44	81	30.4 +/- 19		8	89	40.03 +/- 25.7	
Female lifespan (days) <i>C. capitata</i>	4	31	12 +/- 8.2		7	46	17.5 +/- 11.1		3	42	15.5 +/- 9.8		7	39	13.5 +/- 9.6	

5.3.2 Development to pupal and adult stages

Both *B. dorsalis* and *C. capitata* had similar immature developmental rates on all fruit types (Table 3). Both *B. dorsalis* and *C. capitata* produced the highest mean numbers of pupae, pupae per gram of fruit and mean number of adults on nectarine (Table 2). There were no significant interaction effects between the crop and number of days to pupation [$F_{(3, 24)}=2.1687$, $p=0.11799$]. On all deciduous fruit except apple, days to pupation for *B. dorsalis* and *C. capitata* were similar (Table 3). Larvae of *C. capitata* took significantly longer than those of *B. dorsalis* to pupate on apple at the 0.05% probability level (Table 3). Development to adulthood was faster on nectarine and slowest on apple for both *C. capitata* and *B. dorsalis* (Table 4). Adult emergence was over 90% on all crops for both species, except for *C. capitata* on apple which was at 84% (Table 3). The ratio of male: female flies were about 50:50 for both species on all the fruit types (Table 4).

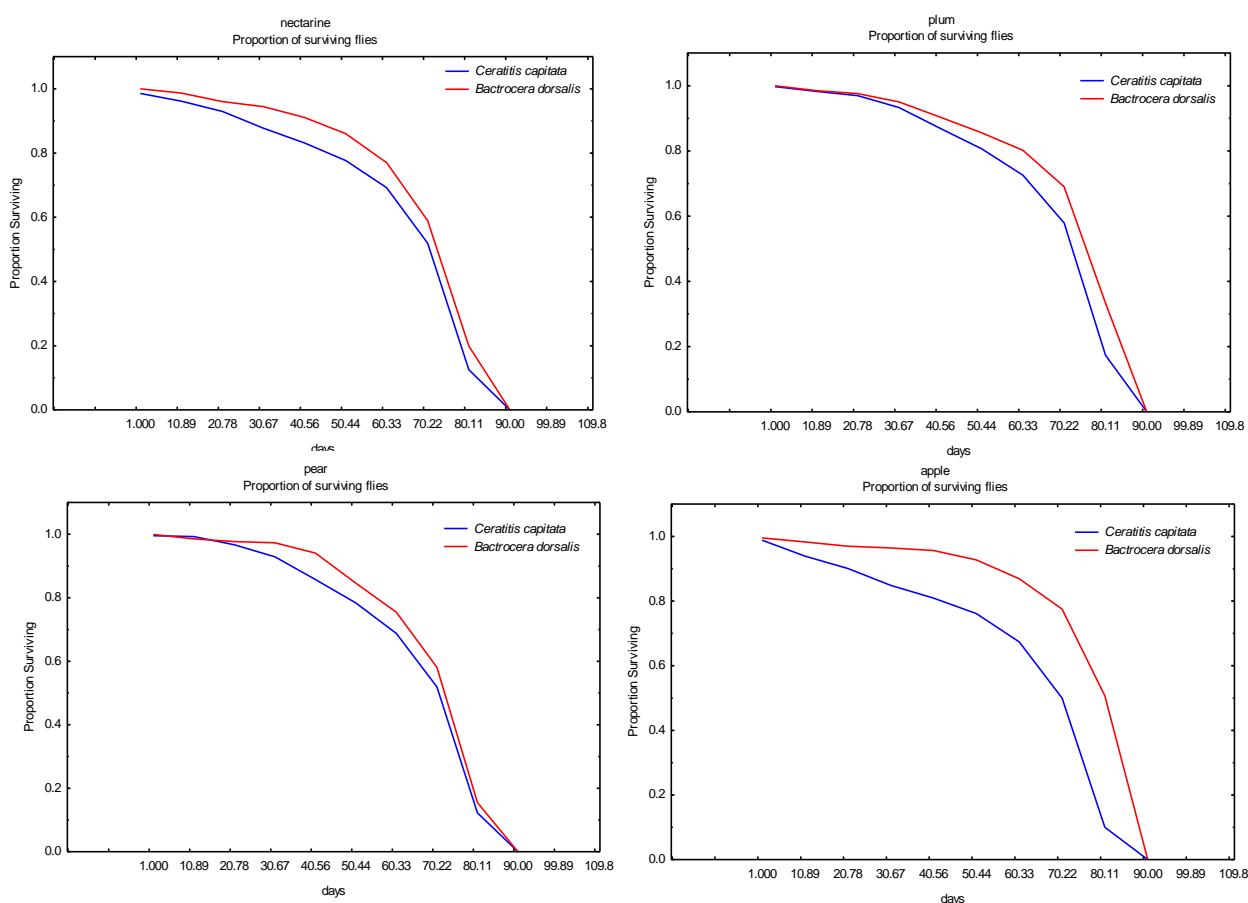


Figure 3. Survivorship curves for *Ceratitis capitata* and *Bactrocera dorsalis* for both sexes on four different deciduous fruit types recorded over 90 days. $p < 0.01$ for all fruit types. Nectarine $Z = -11.8$, Plum $Z = -5.4$, Pear $Z = -7.8$, Apple $Z = -20.7$.

5.3.3 Adult survival

Bactrocera dorsalis adults (males and females) generally lived longer than those of *C. capitata*, irrespective of the fruit types that they developed from (Table 5). *Bactrocera*

dorsalis reared from apple survived longer than those reared on any of the other crops ($Z = -20.7$), (Table 4 and Fig. 3). On all other crops, *B. dorsalis* and *C. capitata* had similar adult survival rates (Table 4 and Fig. 3).

5.3.4 Life Table parameters

Bactrocera dorsalis had a higher net reproductive rate (R_0) on all deciduous fruit tested compared to *C. capitata* (Table 6). The value of R_0 was the lowest for *C. capitata* on apple and highest on plum. For *B. dorsalis*, R_0 was lowest on nectarine and highest on pear. *Ceratitis capitata* had a shorter generation time (T) on all deciduous fruit types tested compared to *B. dorsalis*. T for *C. capitata* was shortest on apple and longest on nectarine and plum. T for *B. dorsalis* was longest on apple and more or less similar for the other fruit types tested. *Ceratitis capitata* had a higher intrinsic rate of increase (r_m) compared to *B. dorsalis* on all fruit types.

Table 5. Life table parameters (net reproductive rate, R_0 ; intrinsic rate of increase r_m ; mean generation time T) for *Ceratitis capitata* and *Bactrocera dorsalis* females on four different deciduous fruit types.

<i>Ceratitis capitata</i>					<i>Bactrocera dorsalis</i>			
Parameter	Nectarine	Plum	Pear	Apple	Nectarine	Plum	Pear	Apple
R_0	187.45	304.25	214.75	33.2	310.4	345.1	684.3	331.35
r_m	0.25	0.25	0.26	0.35	0.15	0.15	0.15	0.12
T	17.89	17.88	17.16	12.86	29.65	29.07	29.07	36.28

There was a significant interaction between fruit*species*sex [$F_{(3, 1424)}=11.356$, $p<0.001$] when analysing the survival of *B. dorsalis* and *C. capitata* adults on the different fruit types (Fig. 4, Table 4). Significantly more flies of *B. dorsalis* than *C. capitata* survived over the 90 day period on all crops, except for males on pear, although this difference between the two species was not significant (Fig. 4, Table 4).

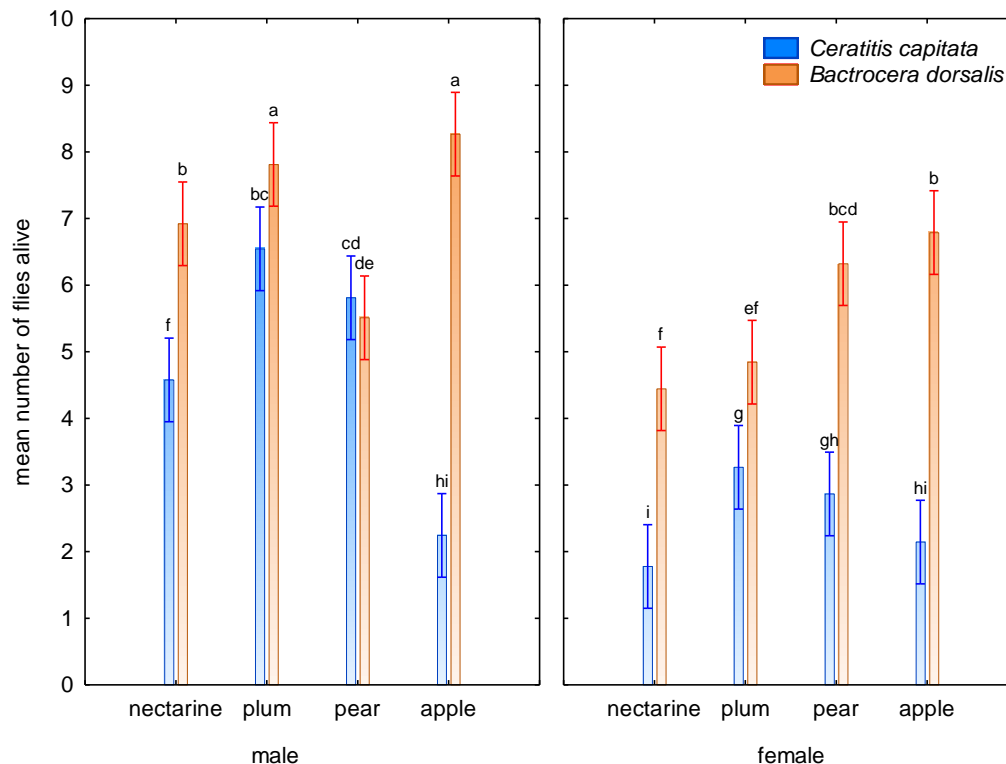


Figure 4. The mean number of live *Ceratitis capitata* and *Bactrocera dorsalis* of both sexes on four different deciduous fruit types recorded daily over 90 days. Vertical bars denote ± 0.95 confidence intervals. Values indicated by the same letter do not differ significantly at $p = 0.05$.

5.4 Discussion

In this study, both *B. dorsalis* and *C. capitata* completed their life cycles successfully on all the deciduous fruit tested, with demographic parameters of the two species differing according to fruit type. *B. dorsalis* was able to survive longer as adults on deciduous fruit, made more oviposition attempts and laid more eggs than *C. capitata*. Immature development and development to adulthood, on the other hand, were more or less the same for *B. dorsalis* and *C. capitata* on the deciduous fruit tested. *Bactrocera dorsalis* had a higher net reproductive rate (R_0), but lower intrinsic rate of increase (r_m) and generation time (T) than *C. capitata* on all deciduous fruit types tested. Survival and development of other fruit fly species have been found to differ between fruit types which are within their host ranges (Hafsi *et al.*, 2016). In surveys in Tanzania, for instance, Mwatawala *et al.* (2009) did not find a high rate of infestation of deciduous fruit by *B. dorsalis*. On apple and peach sampled in Tanzania, *Ceratitis rosa* Karsch was more dominant than *B. dorsalis* (Mwatawala *et al.* 2009) and the authors suggested that the cooler highland areas where apple and peach were grown were more favourable for *C. rosa*. Host fruit was found to influence the demographic parameters of *C. capitata* with regard to the fecundity and survival as well as the r value (intrinsic rate of population increase) and mean regeneration time (Krainer *et al.*, 1987). Rwomushana *et al.* (2008) also found that host fruit influenced the demographic

parameters of *B. dorsalis* when they compared life history parameters of *B. dorsalis* on fourteen cultivated and wild fruit species in Kenya. In this study, generally males of *C. capitata* and *B. dorsalis* lived longer than the con-specific females. This is similar to findings of Papadopoulos *et al.* (2002) on *C. capitata* and Ekesi *et al.* (2006) on *B. dorsalis*. The differences in adult lifespan between males and females could be due to the physiological cost of producing eggs (Vargas & Carey, 1989), also discussed by Carey *et al.* (1995).

Prunus persica (peach and nectarine) and *Prunus domestica* (plum) have been found to be good hosts for *B. dorsalis* (Ye & Liu, 2005) and *C. capitata* (Ovruski *et al.*, 2003; Liquido *et al.*, 1990). Our results confirmed this. In this study lower fecundity of *C. capitata* on nectarine and plum was recorded, which contrasted with the findings of Krainacker *et al.* (1987). The differences between these findings and those of Krainacker *et al.* (1987) could be attributed to the lower temperature ($26^{\circ}\text{C} \pm 1^{\circ}\text{C}$) used in our study compared to 30°C ($\pm 5^{\circ}\text{C}$) in the latter study. McDonald & McInnis (1985) found that fruit size had an influence on the number of eggs produced by *C. capitata*. A higher number of eggs per female were recorded when flies were exposed to whole fruits over 24 hours. A lower number of eggs were produced on plum, the smallest fruit used, over 90 days in these experiments. Small containers with a diameter of 15 mm covered with punctured parafilm were used in the experiments over 90 days; another factor which could have reduced the number of eggs deposited (McDonald & McInnis 1985). The eggs of *B. dorsalis* hatched earlier than those of *C. capitata*, giving the developing *B. dorsalis* larvae a competitive edge over *C. capitata* larvae. Nectarine was the fruit type where both species produced the highest numbers of pupae and larvae in the shortest time.

Malus pumila (apple) was not found to be a good host for *C. capitata* when compared to apricot, peach and orange (Papadopoulos *et al.* 2002). No larvae of *C. capitata* survived in apple during the host demographic studies of Carey (1984). According to the latter author, the flesh of the apple fruit was too firm for the larvae to feed on. Apples are used as egg receptacles in experiments for the culturing of fruit flies on artificial medium (Shelly *et al.*, 2010; Tanga *et al.*, 2015). In the current experiments, *C. capitata* deposited significantly fewer eggs than *B. dorsalis* on apple when comparing the number of eggs deposited by one female over 90 days in small containers. When five females were exposed to whole apples, both species deposited similar and higher numbers of eggs in the fruit. Shelly *et al.* (2010) reported an average number of 26.2 eggs per female *B. dorsalis* over a period on 10 hours on an artificial diet, which is lower than the average number of 39 eggs per female we recorded. The development of pupae and emergence of adults took significantly longer on apple for both *C. capitata* and *B. dorsalis* (Papadopoulos *et al.* 2002). Papadopoulos & Katsoyannos (2002) found that some apple varieties were better hosts for *C. capitata* than

others. In these experiments only Golden Delicious apples were used, so the same experiments should be repeated using different varieties of apples. Apple was suggested as not being a good host for *C. capitata* by Ovruski *et al.* (2003), who did not find any infested apple or pear fruit during their host survey study in Argentina. Hui (2001) and Chen & Ye (2007), on the other hand, described pear as a good host for *B. dorsalis* from field tests and Carey (1984) found that pear was a good host for *C. capitata* in laboratory experiments. These previous findings are in agreement with the results from this study.

Host fruits with longer larval development times represent potential overwintering hosts for fruit flies until suitable environmental conditions are restored (Papadopoulos *et al.*, 2002). Papadopoulos *et al.* (1996) found that apple, as opposed to other fruit such as pear, stay more intact and provide a refuge for larvae that protects them from the elements. In this study, a long period of egg production was found on apple for *B. dorsalis*. Apples could therefore represent ideal bridging hosts for *B. dorsalis* to survive until other fruit become available and suitable environmental conditions are restored.

The demographic parameters of *B. dorsalis* on deciduous fruit is very similar to those on mango (recorded by Ekesi *et al.*, 2007), the preferred host of this fruit fly species (Ekesi *et al.*, 2006). Ekesi *et al.* (2007) compared the demographic parameters of *B. dorsalis* reared on mango to those reared on an artificial diet. They found that larval development takes 10 days under similar rearing conditions as was found in this study and that about 80% of the eggs and pupae emerge. Ekesi *et al.*, 2007 recorded a lower number of eggs (per 10 females) than was recorded on deciduous fruit (per 5 females) in this study. This is an indication that *B. dorsalis* could maintain similar populations on deciduous fruit as on mango under the suitable climatic conditions. The Western Cape of South Africa has a Mediterranean climate, which could be a limiting factor in the establishment of high populations throughout the year (see chapter three) Mean maximum temperatures in the Western Cape of South Africa can exceed 35°C in summer and approach 0°C in the winter (Manrakhan & Addison, 2013) .

The results from this study could assist pest control managers to determine the pest control efforts in areas under different deciduous fruit types. According to the results of these experiments, nectarine and plum were suitable fruit types for *B. dorsalis* and would be able to sustain high populations of the pest. Pear was also found to be suitable fruit type for the fly to complete its life cycle and could be used as an alternative crop when the preferred crops are not available. Apple would possibly mainly be utilized as a refuge crop for initial overwintering. The role of citrus fruit and other fruit types as potential overwintering hosts in the Western Cape should be assessed in future studies.

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Chapter 6

Interspecific competition between *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on deciduous fruit.

6.1 Introduction

Interspecific competition regulates the distribution and abundance of a number of phytophagous insects (Denno *et al.*, 1995, Lopes *et al.*, 2015). The consequences of interspecific competition could be competitive exclusion, displacement and fitness reduction of the competing species (Denno *et al.*, 1995, Duyck, 2004, Ekesi *et al.*, 2009). Factors such as host plants are highly influential in mediating interspecific competition among phytophagous insects (Denno *et al.*, 1995). Fruit flies (Diptera: Tephritidae) are distributed globally and are mostly phytophagous (White & Elson-Harris, 1992). The larvae of about 35% of phytophagous species attack fruit that include fruit crops of economic importance (White & Elson-Harris, 1992). A few of these frugivorous fruit fly pests are highly polyphagous, attacking a wide range of species in different plant families, and often have overlapping host ranges (White & Elson-Harris, 1992). In environments shared between different polyphagous fruit fly species using similar hosts, competitive interactions between the latter species are likely to occur. Female choice and larval performance are the two most important factors that determine the suitability of a plant as a host for tephritid species (Jaenike, 1990; Ravigné *et al.*, 2009).

Deciduous fruit in South Africa is mainly cultivated in the Western Cape Province, with *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* s.l. Karsch being the most economically important fruit fly species (Barnes *et al.* 2007, Manrakhan & Addison 2013) in the province. *Ceratitis rosa* s.l. was recently split in two species: *C. rosa* s.s. Karsch and *Ceratitis quilicii* De Meyer, Mwatawala, Copeland and Virgilio (De Meyer *et al.*, 2016) with *C. quilicii* being the only one of the two present in the Western Cape (Virgilio *et al.*, 2013; Karsten *et al.*, 2016). *Ceratitis capitata* was recorded as the most widespread *Ceratitis* pest species in the Western Cape (De Villiers *et al.*, 2013) as well as the dominant species on deciduous fruit in the region (Manrakhan & Addison, 2013). *Bactrocera dorsalis* (Hendel), of Asian origin, is a new fruit fly pest in South Africa and is currently present in the northern and north-eastern parts of the country (Manrakhan *et al.*, 2015). The pest is still absent in other areas of the country including the Western Cape Province of South Africa. A number of regions in South Africa, including the coastal regions of Western Cape, were however deemed suitable for establishment of *B. dorsalis* based on a recent climatic model (De Villiers *et al.*, 2016). Both *C. capitata* and *B. dorsalis* are highly polypagous pests with overlapping host ranges (White & Elson-Harris, 1992; De Meyer *et al.*, 2002; Clarke *et al.*, 2005).

Bactrocera dorsalis is known to be a large, dominant fly, able to out-compete *Ceratitis* species in Africa (Duyck *et al.*, 2006, Mwatawala *et al.*, 2006, Ekesi *et al.*, 2009). The aggressive behaviour displayed by adult flies combined with the large size and other demographic characteristics makes it possible for the fly to invade new areas using the hierarchical mode of competition (Duyck *et al.*, 2004). After invading Tahiti in 1996, *B. dorsalis* also displaced other *Bactrocera* species such as *Bactrocera kirki* (Froggatt) and *Bactrocera tryoni* (Froggatt) (Leblanc *et al.*, 2013). After invading Hawaii, *B. dorsalis* displaced the *Ceratitis* species that were established on the island (Keiser *et al.*, 1974) in the lowland areas of the island. Host plant played an important role in the ability of *B. dorsalis* to out-compete *C. capitata* in Hawaii, since *C. capitata* was able to persist in the lowlands of Hawaii on coffee (Vargas *et al.*, 1995), an ancestral host of *C. capitata* (Malacrida *et al.*, 1992). After being detected in the northern region of Swaziland in January 2013 for the first time, *B. dorsalis* is now the dominant species over *C. capitata*, *C. cosyra* and *C. brevidens*, which were the most abundant fruit fly species detected on mango before the invasion of *B. dorsalis* (Magagula & Nzima, 2017). Migani *et al.* (2014) showed that *B. dorsalis* females are more prone to accept new and less preferred hosts, because of its high and continuous egg production. Ekesi *et al.* (2009) showed that in co-infestations of mango fruits, *C. cosyra* larvae were negatively affected by *B. dorsalis* due to their highly effective ability to compete for the available resources.

Exploitative competition by tephritid larvae in fruit can lead to lower pupal weight and impact negatively on larval survival (Duyck *et al.*, 2008), with the resulting influence on the net reproductive rate. Competition between species can also be due to differences in life history traits or behaviour of the species through the use of interference strategies (Duyck *et al.*, 2004). Vargas *et al.* (2000) categorize *B. dorsalis* as a "K-selected" (Pianka, 1970) species, since it is a larger fly with later onset of reproduction. *Ceratitis capitata*, on the other hand, falls in the "r-selected" species due to its small size and earlier onset of reproduction (Malacrida *et al.*, 2007, Vargas *et al.*, 2000). The K selective trait favours the invasive potential of *B. dorsalis* in favourable habitats (Malacrida *et al.*, 2007). Based on results of more recent studies in Africa, *B. dorsalis* was described as possessing characteristics of both K and r strategies, because of its aggressive character and ability to adapt to new environments as well as its high fecundity (Ekesi *et al.*, 2006). When considering the demographic characteristics of the adult flies, Rwomushana *et al.* (2009) found that *B. dorsalis* reached sexual maturity within seven days and that females produced most of their eggs between 8 and 22 days after reaching maturity. One mature female can produce over 1000 eggs in her lifetime, of which 55% will develop to adults. If conditions are suitable, a population *B. dorsalis* can increase by 11% per day and double after six days (Ekesi *et al.*,

2006). Females of *C. capitata* reach sexual maturity within 4 days (Arita, 1982) and produced most of their eggs between 4 and 20 days (Papadopoulos *et al.*, 2002) after achieving maturity. Under ideal conditions, one mature female can produce from 10 - 22 eggs per day and as many as 800 eggs during her lifetime (usually about 300). *Ceratitis capitata* can complete its life cycle between 21-30 days (Steck, 2002).

Host plants play an important role in the ability of fruit fly species to survive and disperse (Malacrida *et al.*, 2007). In the northern parts of South Africa, *B. dorsalis* has so far been recorded on a few hosts (Theron *et al.*, 2017). Grové *et al.* (2017) reared fruit flies from various indigenous fruits in the Limpopo and Mpumalanga provinces and found that *B. dorsalis* only emerged from marula fruit (*Sclerocarya birrea* (A.Rich.) Hochst. (Anacardiaceae)) out of a range of 28 plant species. *Ceratitis capitata* emerged from 12 of 28 indigenous plant species tested (Grové *et al.*, 2017).

Literature on the development of *B. dorsalis* on deciduous fruit is not readily available. White & Elson-Harris 1992 listed *Prunus persica* (L.) Batsch, (Nectarine), *Prunus domestica* L., (Plum), *Malus domestica* Borkh., (Apple) and *Pyrus communis* L., (Pear), as host plants for *B. dorsalis*. Ye & Liu (2005) found that apple was a less preferred host for *B. dorsalis* in China and that pear was not infested as frequently as peach and could not support high numbers of *B. dorsalis*. *Ceratitis capitata*, on the other hand, was described as a serious pest of deciduous fruit by Thomas *et al.* (2001).

In the possible event of an introduction of *B. dorsalis* in the Western Cape, it would be important to predict the likely interactions between *B. dorsalis* and *C. capitata* on deciduous fruit. *Ceratitis capitata* was chosen as the tested competitor because of its current dominance on deciduous fruit in the potentially invaded area. Such studies will be important in planning response actions against *B. dorsalis* should there be an invasion of this pest in the Western Cape.

The aim of this study was to quantify adult and larval interactions between *B. dorsalis* and *C. capitata* on four deciduous fruit types which are mainly cultivated in the Western Cape Province, South Africa: *Prunus persica* (L.) Batsch, (Nectarine), *Prunus domestica* L., (Plum), *Malus domestica* Borkh., (Apple) and *Pyrus communis* L., (Pear).

6.2 Materials and Methods

All experiments were conducted in a quarantine insectary at the Plant Quarantine Station of the Department of Agriculture, Forestry and Fisheries (DAFF) in Stellenbosch, South Africa. The conditions in the insectary were controlled at 26°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity with a 12h light/12h dark cycle. One hour dawn and dusk was created by connecting 40W bulbs to a time switch. A 40W bulb was switched on simulating 1 h dawn and 1 h dusk every day within the 12 h light cycle.

6.2.1 Insect materials

Bactrocera dorsalis was reared in the Insect Quarantine Facility of the Agricultural Research Council in Stellenbosch. They were reared at 27°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in Perspex™ cages (30 x 30 x 40 cm, 36 l) with a fabric sleeve under natural light conditions and provided with perforated apple halves for oviposition as well as water and a mixture of sugar and yeast as food (Barnes *et al.* 2007). The culture was started from infested guavas collected near Thohoyandou in Limpopo Province during March 2014 and wild flies from the same area was added once a year. The perforated apple halves provided for oviposition were removed every two days. Larvae were reared on an artificial larval rearing medium (Barnes *et al.* 2007) with the addition of 100g carrot powder per kg of mix and kept in separate containers on vermiculite at 27°C ($\pm 1^\circ\text{C}$) for pupation. The vermiculite was sifted to remove the pupae, which were placed in honey jars marked with the date collected. The flies that emerged were released into cages marked with the day of emergence. The flies used in the experiments were 14 (± 1) days old.

Ceratitis capitata was reared in the insect rearing facility at the Welgevallen experimental farm, Stellenbosch University. They were reared at 25°C ($\pm 1^\circ\text{C}$) and 70% ($\pm 5\%$) humidity in Perspex™ cages (800 mm³) under 12h light/12h dark conditions and provided with apples for oviposition as well as water and a mixture of sugar and yeast hydrolysate as food (Barnes *et al.* 2007). Pupae to start the colony were obtained from colonies held at Citrus Research International (CRI) in Nelspruit, Mpumalanga province, South Africa. The perforated apple halves provided for oviposition were removed every two days. Larvae were reared on an artificial larval rearing medium (Barnes *et al.* 2007) and kept in separate containers on vermiculite at 25°C ($\pm 1^\circ\text{C}$) for pupation. The vermiculite was sifted to remove the pupae, which were placed in honey jars marked with the date collected. The flies that emerged were released into cages marked with the day of emergence. The flies used in the experiments were 7 (± 1) days old.

6.2.2 Deciduous fruit tested

Four deciduous fruit types were used in all tests: *Prunus persica* (L.) Batsch, Nectarine “Arctic Star” and Nectarine “Mongreb”; *Prunus domestica* L., Plum “Fortune”; *Malus domestica* Borkh., Apple “Golden delicious”; *Pyrus communis* L., Pear “Packham”. Tests were carried out between December 2016 and June 2017.

Fruit was purchased the day before it was needed in a shop selling fruit grown under good agricultural practices and left at 25°C overnight before using in the experiments.

6.2.3 Interspecific interactions: adults on host fruits

Bactrocera dorsalis and *C. capitata* females were placed separately or jointly in separate 19 x 15 x 16 cm 4.5 l aerated insect cages and provided with water and a mixture of sugar and

yeast as food. Each cage contained a total of 30 flies. One test fruit was placed in each cage for 24 hours. Each test fruit was weighed before placement into the cage.

The following ratios were used, with a total of 30 flies per cage: *B. dorsalis*: *C. capitata*, 1:4, 4:1, 1:1. Cages containing only *B. dorsalis* and only *C. capitata* were included and 15 males of each species were added in the same ratio as the females.

The flies were exposed to one fruit type at a time. Observations on fly behaviour were done in the morning at 08:00, midday at 13:00 and afternoon at 18:00. Number of flies (per species) ovipositing on fruits and incidence of aggression between species were recorded.

At the end of each test day, all fruits from the cages were incubated in separate containers with respect to fly groupings. Fruits were placed on a vermiculite layer in a 2 l plastic container with cloth in the lid for aeration and left to incubate at 26°C ($\pm 1^\circ\text{C}$). The vermiculite was sifted daily after 7 days and the numbers of pupae were recorded daily until no more pupae were produced. The pupae were placed in honey jars with aerated lids for the adults to emerge. Pupae and adult flies were counted daily, and the adults identified and sexed. The experiment was replicated four times.

6.2.4 Interspecific interactions: larval co-infestation of fruits

Newly emerged larvae of *B. dorsalis* and *C. capitata* were placed in the same fruit. The following ratios were used, with a total of 40 larvae per fruit: *B. dorsalis*: *C. capitata*, 1:1, 1:3 and 3:1. Fruit containing only *B. dorsalis* and only *C. capitata* larvae were included.

Two cones (approx. 8 mm in diameter and 1 cm deep) were cut from opposing sides of each test fruit and 20 one (± 1) day old larvae were placed in each cone. The removed piece of fruit was placed back as a lid. A total of 40 larvae were introduced at a time. Infested fruits were kept at 25°C.

The fruit were placed on vermiculite in individual 2 l plastic boxes with cloth in the lid for aeration until pupation. The vermiculite was sifted daily after seven days and the numbers of pupae were recorded daily until no more pupae were formed. The pupae were placed in honey jars with aerated lids for the adults to emerge. Adult emergence was recorded daily, noting the number of males and females emerging every day. The experiment was replicated five times for each fruit type.

6.2.5 Statistical analysis

The following main effects were analysed using factorial ANOVA: number of pupae, number of pupae per gram of fruit, percentage hatch, number of flies, number of days to pupation, number of adults of both species that emerged, number of incidences of aggression between the two species, number of times the species oviposited on the different fruit types, and the percentage species composition of the fly species on each fruit type. Interactions between

the main effects and independent variables (fruit type, fruit fly species and ratios of *B. dorsalis* to *C. capitata*) were also determined. Data collected over time was amalgamated into 7 day periods (weeks) for clarity of the graphs and analysed using Repeated Measures ANOVA analyses. Mean values were compared using least significant differences (LSD) post-hoc tests using Statistica 7.0 (Statsoft, Tulsa, USA).

6.3 Results

6.3.1 Adult competition on the host fruit

Bactrocera dorsalis and *C. capitata*, when evaluated separately, infested nectarine and pear at more or less the same rates based on the number of pupae reared from the fruit (Table 1). On plum, *B. dorsalis* produced significantly more pupae and adults [both pupae ($F_{(3, 48)}=18.705$, $p<0.001$) and adults, ($F_{(3, 48)}=11.463$, $p<0.001$) from the fruit compared to *C. capitata* (Table 1). On apple, *B. dorsalis* also produced numerically more pupae and adults than *C. capitata*, but these differences were only statistically significant for the pupae ($p<0.05$) (Table 1). Both species produced the lowest number of pupae on pear ($p<0.05$) (Table 1). Pupal development of *B. dorsalis* was also significantly slower on pear whilst for *C. capitata*, pupal development was significantly slower on apple.

In competition experiments where *B. dorsalis* dominated, *B. dorsalis* had the highest degree of infestation (adults per fruit) on all deciduous fruit (Fig. 1). In the competition experiments where *C. capitata* dominated, *B. dorsalis* still had the higher degree of infestation in nectarine and apple (Fig. 1). When *B. dorsalis* and *C. capitata* were exposed to fruit in equal adult numbers, *B. dorsalis* dominated in plum and apple whilst *C. capitata* dominated on nectarine and pear (Fig. 1). Significantly more pupae were produced per gram of fruit on nectarine in all treatments and the least number of pupae per gram of fruit were produced on pear ($p<0.05$) (Table 1). Pupal developmental times differed significantly for all species in the competition experiments [$F_{(3, 48)}=125.51$, $p<0.001$] (Table 2) on the four fruit types tested. Pupal development was fastest on nectarine and the slowest on apple (Table 2).

There were no significant interaction effects between fruit type and treatment when analysing the numbers of pupae produced on the different fruit types in the competition treatments [$F_{(6, 48)}=0.68412$, $p=0.66322$], or the control treatments [$F_{(3, 32)}=2.5838$, $p=0.07044$]. There were no significant interaction effects between fruit and fruit fly species when analysing the mean number of flies that emerged in the control experiments [$F_{(3, 32)}=2.8146$, $p=0.05487$]. There were also no significant interaction effects between fruit and treatment between the mean numbers of *C. capitata* and *B. dorsalis* reared from the adult competition experiments [$F_{(6, 48)}=2.2388$, $p=0.05519$]. There was a significant interaction between weeks, species and fruit type when analysing the mean number of adults of both species that emerged on all fruit types for the competition treatments [$F_{(12, 928)}=16.496$,

$p < 0.001$] (Table 2). Both species produced the highest number of adults on nectarine during week one whilst on pear this was during week three.

Table 1. Main effects of adult competition between *Ceratitis capitata* and *Bactrocera dorsalis* on pupae and adults reared from four different deciduous fruit types. In each section and column, means followed by the same letters in the column are not significantly different at 0.05% probability level. The main effect means are indicated in bold.

Treatment*	Mean number of pupae					Mean no of pupae/gram of fruit					Mean % adult emergence					Mean number of flies				
	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
A (1:4)	125 ^{abc}	66 ^{bcd}	44 ^{cd}	124 ^{abc}	89^a	1.2 ^{abc}	0.5 ^{cd}	0.2 ^d	0.9 ^{bcd}	0.7^a	60 ^{bcd}	86 ^a	52 ^d	76 ^{abc}	68^a	73 ^{abc}	56 ^{bc}	22 ^c	93 ^{ab}	61^a
B (4:1)	179 ^a	135 ^{ab}	33 ^d	109 ^{abcd}	114^a	1.7 ^a	1.1 ^{abc}	0.2 ^d	0.8 ^{bcd}	1.0^a	63 ^{bcd}	86 ^a	74 ^{abc}	54 ^{cd}	69^a	116 ^a	116 ^a	26 ^c	59 ^{bc}	79^a
C (1:1)	152 ^a	108 ^{abcd}	33 ^d	147 ^{ab}	110^a	1.5 ^{ab}	0.9 ^{bcd}	0.2 ^d	1.0 ^{abc}	0.9^a	86 ^a	82 ^{ab}	70 ^{abcd}	62 ^{bcd}	75^a	126 ^a	91 ^{ab}	21 ^c	91 ^{ab}	83^a
means	152^a	103^b	36^c	126^{ab}		1.5^a	0.9^b	0.2^c	0.9^b		70^b	85^a	65^b	64^b		105^a	88^a	23^b	81^a	
Only <i>C. capitata</i>	146 ^{abc}	100 ^{bcd}	42 ^{de}	80 ^{cde}	92^a	1.5 ^{ab}	0.8 ^{bc}	0.2 ^{cd}	0.6 ^{cd}	0.8^a	86 ^a	81 ^{ab}	79 ^{ab}	60 ^{ab}	73^a	121 ^{abc}	83 ^{bcd}	32 ^{de}	55 ^{cde}	73^a
Only <i>B. dorsalis</i>	177 ^{ab}	187 ^a	8 ^e	194 ^a	142^a	1.6 ^{ab}	1.6 ^a	0.05 ^d	1.4 ^{ab}	1.1^a	78 ^{ab}	84 ^a	71 ^{ab}	70 ^a	79^a	145 ^{ab}	155 ^a	6.4 ^e	115 ^{abc}	106^a
means	162^a	143^a	25^b	137^a		1.5^a	1.2^{ab}	0.1^c	1.0^b		82^a	82^a	75^{ab}	65^a		133^a	119^a	19^b	85^a	

* A: 20% *Bactrocera dorsalis*:80% *Ceratitis capitata* (1:4), B: 80% *Bactrocera dorsalis*:20% *Ceratitis capitata* (4:1) and C: 50% *Bactrocera dorsalis*:50% *Ceratitis capitata* (1:1)

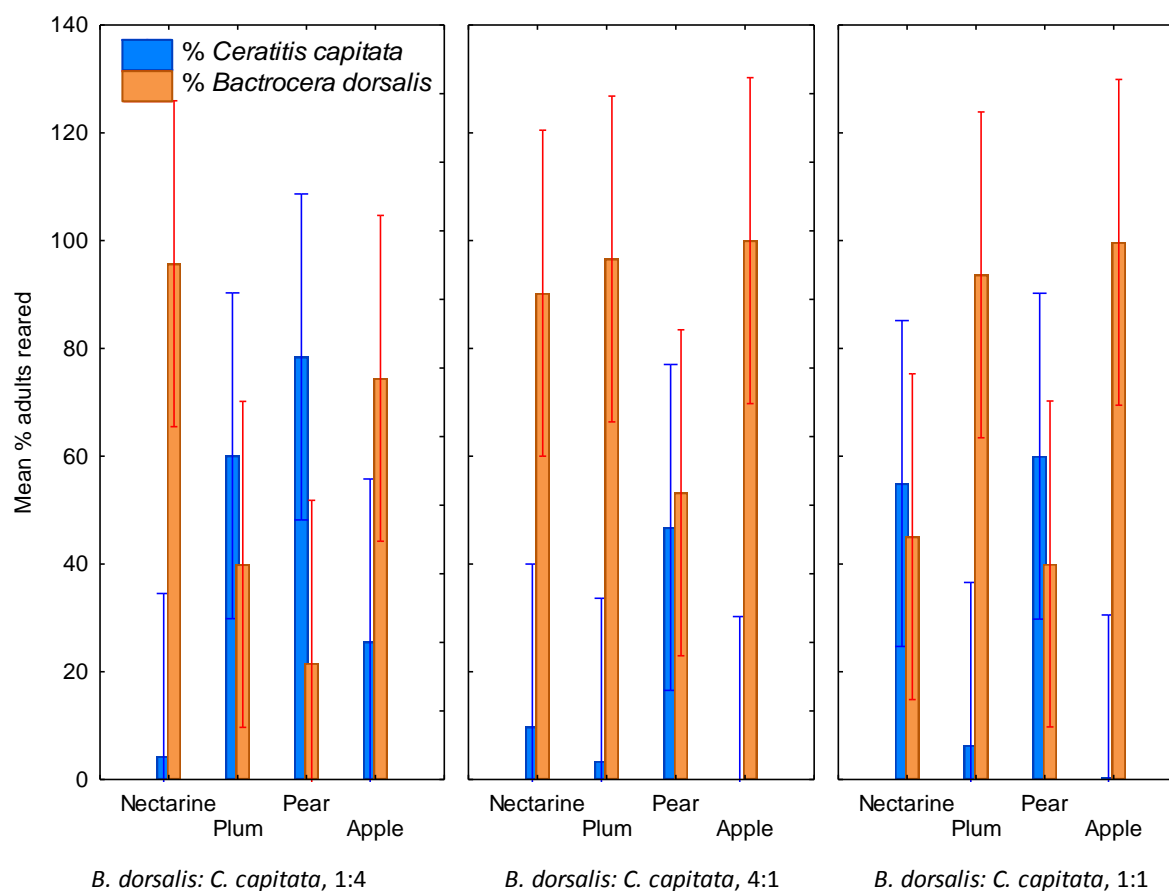


Figure 1. Mean percentage *Ceratitis capitata* and *Bactrocera dorsalis* reared from four different deciduous fruit types in the adult competition experiment. \pm Vertical bars denote 0.95 confidence intervals.

When tested separately, the highest number of oviposition events was recorded on nectarine for both species (Fig. 2). *Bactrocera dorsalis* oviposited more frequently on plum and apple than *C. capitata* ($p < 0.05$). Oviposition was recorded mostly in the morning for both species. During the afternoon and dusk, *B. dorsalis* had significantly more oviposition events than *C. capitata* ($p < 0.05$). There was an interaction between the species, fruit type and treatment when analysing the number of times oviposition was recorded (Fig 2) [$F_{(12, 560)} = 4.0720$, $p < 0.001$]. *Bactrocera dorsalis* and *C. capitata* oviposited most frequently on nectarine. *Bactrocera dorsalis* oviposited more frequently on plum and apple than *C. capitata* ($p < 0.05$). The interaction between species and time was not significant when analysing the oviposition behaviour for all the treatments [$F_{(2, 480)} = 0.62921$, $p = 0.53345$].

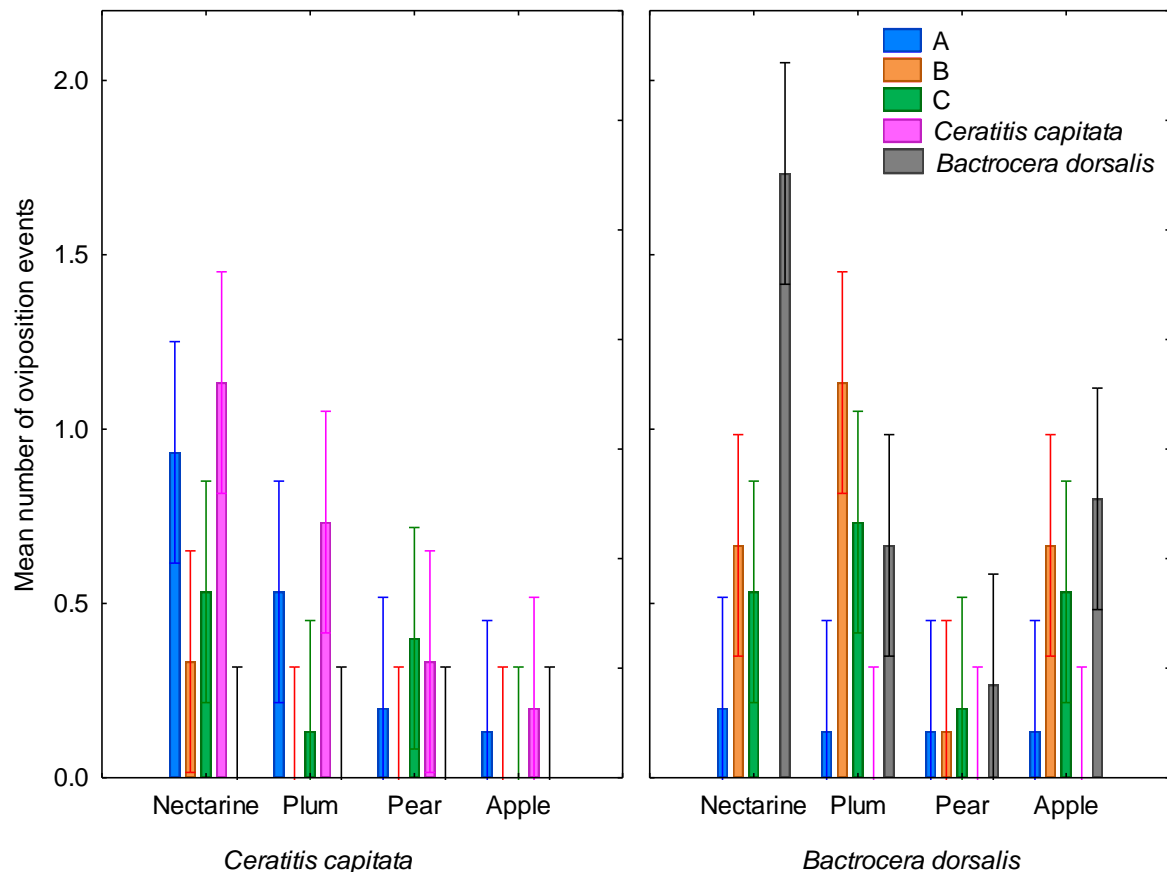


Figure 2. The mean number of oviposition events recorded for *Ceratitis capitata* and *Bactrocera dorsalis* on the four different deciduous fruit types in all the treatments. A: 20% *Bactrocera dorsalis*: 80% *Ceratitis capitata* (1:4), B: 80% *Bactrocera dorsalis*: 20% *Ceratitis capitata* (4:1) and C: 50% *Bactrocera dorsalis*: 50% *Ceratitis capitata* (1:1). \pm Vertical bars denote 0.95 confidence intervals.

When looking at the behaviour of the flies, no aggressive behaviour was recorded for *C. capitata* (Fig. 3). Only *B. dorsalis* displayed aggressive behaviour (Fig. 3) amongst themselves and not towards *C. capitata*. There was a significant interaction between treatment and time when analysing aggressive behaviour [$F_{(8, 585)}=3.0698$, $p=0.00214$]. The most significant incidences of aggression were recorded in treatments B (20% *C. capitata*: 80% *B. dorsalis*) and E (only *B. dorsalis*) ($p<0.05$) and the flies displayed their most aggressive behaviour during the day (morning and noon) ($p<0.05$).

Table 2. Main effects of adult competition between *Ceratitis capitata* and *Bactrocera dorsalis* on days to pupation and adult emergence as well as numbers of emerged adults of each species from four different deciduous fruit types. In each section and column, means followed by the same letters in the column are not significantly different at 0.05% probability level. The main effect means are indicated in bold.

	Mean no of days to pupation					Mean no of days to adult emergence					Mean no of adult <i>Ceratitis capitata</i>					Mean no of adult <i>Bactrocera dorsalis</i>				
Treatment*	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
A (1:4)	8 ^{cd}	9.8 ^c	12.2 ^b	17.8 ^a	11.9^a	16.9 ^e	18.8 ^d	22.5 ^{bc}	24.1	20.2^a	2 ^c	34.6 ^{ab}	20.4 ^{bc}	26 ^{abc}	20.7^a	70.6 ^{ab}	21.4 ^{bc}	1.6 ^c	67 ^{ab}	40^b
B (4:1)	7.8 ^d	9 ^{cd}	12.6 ^b	17.2 ^a	11.7^a	16 ^e	18.3 ^d	23.4 ^{abc}	25.2	20.1^a	2.4 ^c	3.6 ^c	6.4 ^c	0 ^c	3.1^b	113.4 ^a	113 ^a	19 ^{bc}	60 ^{abc}	76.1^a
C (1:1)	7.6 ^d	9 ^{cd}	13 ^b	16.8 ^a	11.6^a	16.4 ^e	18.6 ^d	21.8 ^c	24.2	19^c	47.2 ^a	2.2 ^c	18.6 ^{bc}	0.2 ^c	17.1^a	79.2 ^{ab}	89.2 ^a	2.8 ^c	91 ^a	65.5^{ab}
means	7.8^d	9.3^c	12.6^b	17.3^a		16.5^d	18.6^c	22.6^b	24.4^a		17.2^a	13.5^a	15.1^a	8.7^a		87.7^a	74.5^a	7.9^b	72.1^a	
Only <i>C. capitata</i>	8.4 ^e	9.4 ^e	13.6 ^d	19 ^a	12.6^a	15.8 ^f	19.1 ^d	22.3 ^c	28.3 ^a	21.4^a										
Only <i>B. dorsalis</i>	8.2 ^e	9 ^e	15.4 ^c	17.2 ^b	12.5^a	16.9 ^{ef}	18.1 ^{de}	25.1 ^b	25.3 ^b	21.3^a										
means	8.3^c	9.2^c	14.5^b	18.1^a		16.3^d	18.6^c	23.6^b	26.8^a											

* A: 20% *Bactrocera dorsalis*:80% *Ceratitis capitata* (1:4), B: 80% *Bactrocera dorsalis*:20% *Ceratitis capitata* (4:1) and C: 50% *Bactrocera dorsalis*:50% *Ceratitis capitata* (1:1).

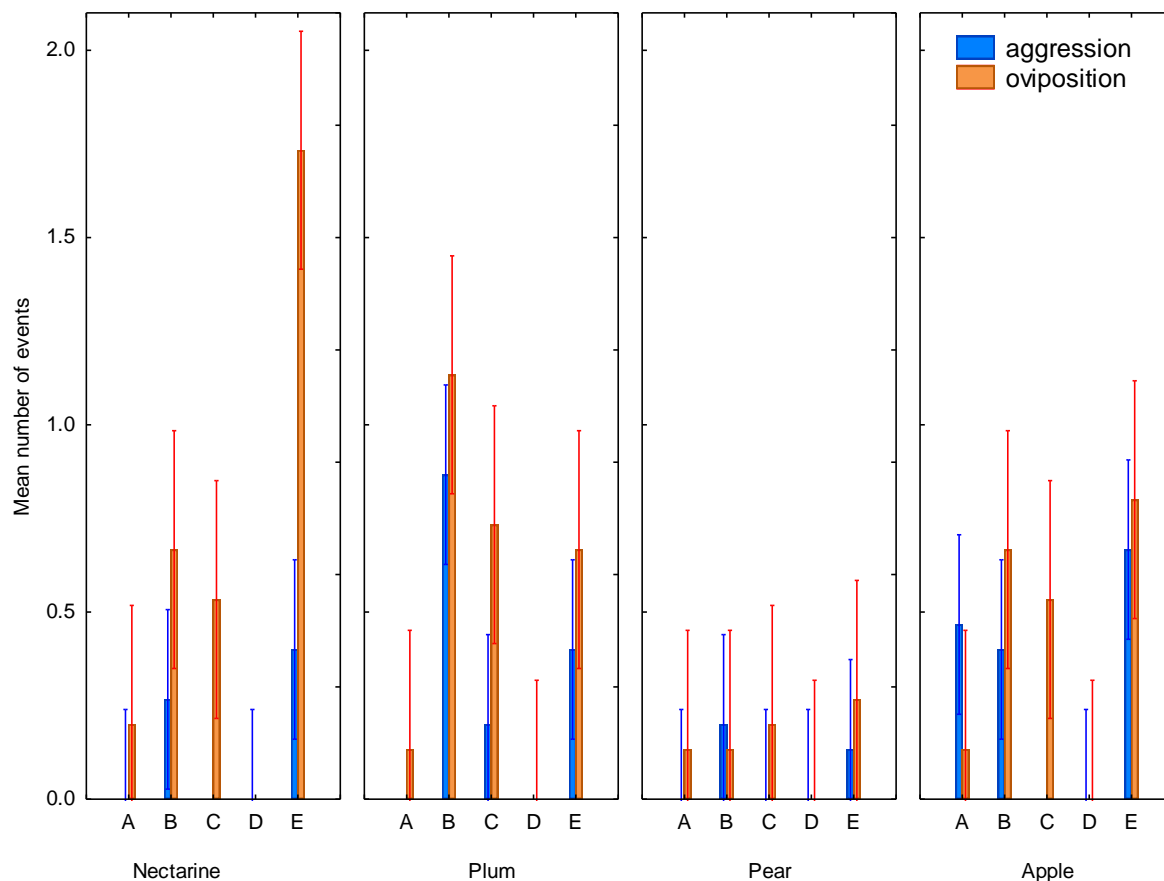


Figure 3. The mean number aggression events and oviposition recorded for *Bactrocera dorsalis* on four different fruit types in all the treatments. A: 20% *Bactrocera dorsalis*: 80% *Ceratitis capitata* (1:4), B: 80% *Bactrocera dorsalis*: 20% *Ceratitis capitata* (4:1), C: 50% *Bactrocera dorsalis*: 50% *Ceratitis capitata* (1:1), D: *Ceratitis capitata* and E: *Bactrocera dorsalis*. \pm Vertical bars denote 0.95 confidence intervals.

The number of oviposition events by *B. dorsalis* correlated well with the mean number of aggressive interactions by *B. dorsalis* in most instances (Fig. 3). There was an interaction between species, fruit type and treatment when analysing the mean number of oviposition and aggression events for *B. dorsalis* [$F_{(24, 1118)}=2.4161$, $p=0.00016$].

6.3.2 Larval competition in the host fruits

In infestation experiments, neonate larvae of both *C. capitata* and *B. dorsalis* were able to complete development to adulthood in all deciduous fruit types tested (Table 3). There was no interaction for either *B. dorsalis* nor *C. capitata* between fruit type and fruit fly species on the numbers of pupae [$F_{(3, 32)}=0.81253$, $p=0.49639$] or adults [$F_{(3, 32)}=2.8146$, $p=0.05487$] reared when they were separately inoculated in fruit. *Ceratitis capitata* generally produced more pupae and adults per fruit compared to *B. dorsalis* on all fruit types (Table 4).

Table 3. Main effects of the competition of the larvae of *Ceratitis capitata* and *Bactrocera dorsalis* on numbers of pupae and adults reared as well as percentage larval and pupal survival on four different deciduous fruit types. In each section and each column, means followed by the same letters in the column are not significantly different at 5% probability level. The main effect means are indicated in bold.

Treatment*	Mean number of pupae					Mean % larvae pupated					Mean % adult emergence					Mean number of flies				
	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
A (1:1)	19 ^{de}	26 ^{bc}	29 ^{ab}	16 ^e	23^b	49 ^{de}	65 ^{bc}	72 ^{ab}	41 ^e	57^b	71 ^{cd}	88 ^{abc}	73 ^{bc}	84 ^{abc}	79^{ab}	15,4 ^{cde}	22,8 ^b	21,2 ^{bc}	14 ^{be}	18,4^b
B (1:3)	22 ^{cde}	35 ^a	29 ^{ab}	20 ^{cde}	26^a	55 ^{cd}	87 ^a	72 ^{ab}	50 ^{cde}	66^a	83 ^{abc}	84 ^{abc}	75 ^{abc}	92 ^a	84^a	18,2 ^{bcd}	29,2 ^a	21,4 ^{bc}	18,4 ^{bcd}	21,8^a
C (3:1)	17 ^e	25 ^{bcd}	23 ^{bcd}	17 ^e	21^b	43 ^e	63 ^{bcd}	59 ^{bcd}	43 ^e	52^b	54 ^d	75 ^{abc}	77 ^{abc}	91 ^{ab}	74^b	9,4 ^e	18,4 ^{bcd}	18 ^{bcd}	15,4 ^{cde}	15,3^c
means	20^b	29^a	27^a	18^b		49^b	72^a	68^a	45^b		69^c	83^{ab}	75^b	89^a		14,3^b	23,5^a	20,2^a	15,9^b	
Only <i>C. capitata</i>	38 ^a	39 ^a	32 ^{ab}	26 ^{bc}	34^a	96 ^a	97 ^a	81 ^{ab}	66 ^{bc}	85^a	86 ^{ab}	94 ^a	88 ^{ab}	87 ^{ab}	88^a	33 ^{ab}	36 ^a	28 ^{bc}	23 ^{cd}	30^a
Only <i>B. dorsalis</i>	30 ^{bc}	28 ^{bc}	25 ^{bc}	24 ^c	27^b	75 ^{bc}	70 ^{bc}	63 ^{bc}	59 ^c	67^b	60 ^c	81 ^{ab}	77 ^b	83 ^{ab}	75^b	17 ^d	23 ^{cd}	19 ^d	19 ^d	20^b
means	34^a	33^a	29^{ab}	25^b		85^a	83^a	72^{ab}	63^b		73^b	88^a	82^a	85^a		25^b	30^a	24^b	21^b	

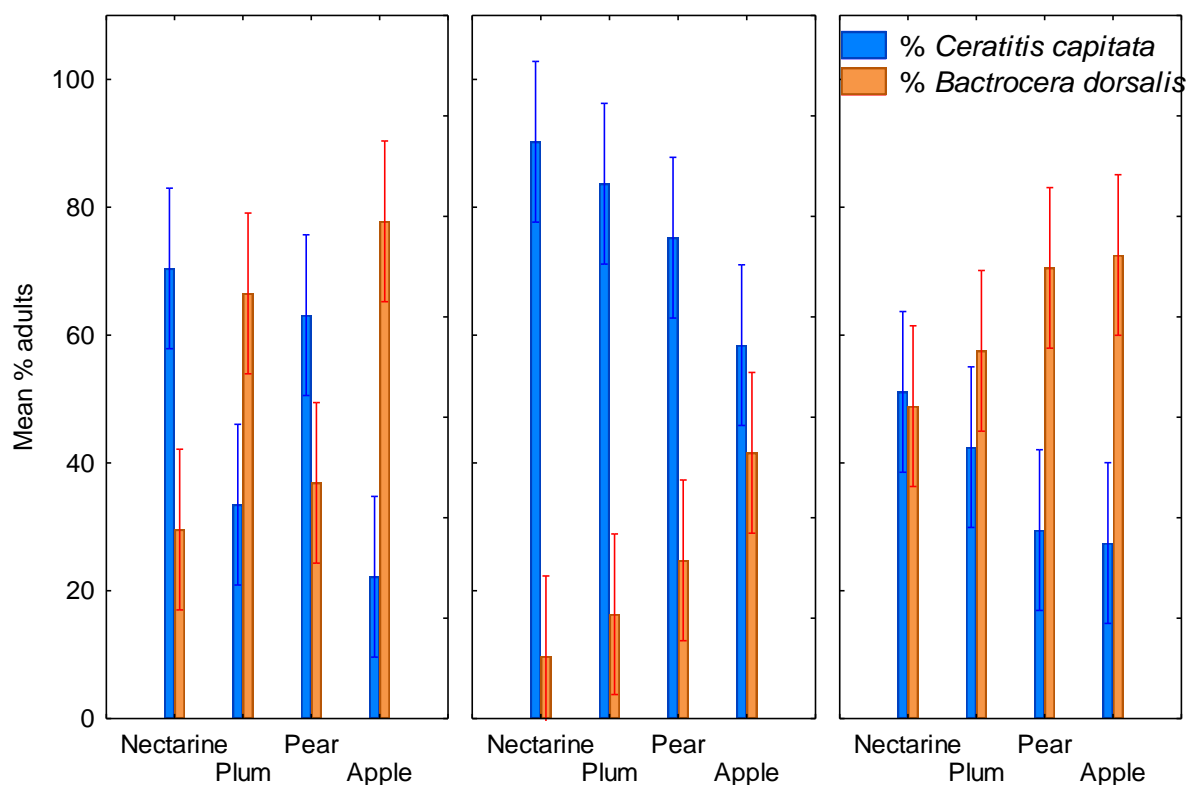
*A: 50% *Bactrocera dorsalis*: 50% *Ceratitis capitata* (1:1), B: 25% *Bactrocera dorsalis*: 75% *Ceratitis capitata*: (1:3) and C: 75% *Bactrocera dorsalis*: 25% *Ceratitis capitata*: (3:1).

Table 4. Main effects of the competition of the larvae between *Ceratitis capitata* and *Bactrocera dorsalis* on days to pupation, adult emergence and emerged adults on four different deciduous fruit types. In each section and each column, means followed by the same letters in the column are not significantly different at 5% probability level. The main effect means are indicated in bold.

Treatment*	Mean no of days to pupation					Mean no of days to adult emergence					Mean number of adult <i>Ceratitis capitata</i>					Mean number adult <i>Bactrocera dorsalis</i>				
	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means	Nectarine	Plum	Pear	Apple	means
A (1:1)	4 ^e	8.4 ^d	7 ^{cd}	7.4 ^{bc}	6.7^a	15.1 ^{cd}	18.3 ^{ab}	18.2 ^{ab}	17.2 ^{ab}	17.2 ^{ab}	11 ^{cd}	7.8 ^{de}	12.8 ^{bc}	3.4 ^f	8.7^b	4.4 ^{de}	15 ^a	8.4 ^{bcd}	10.6 ^{bc}	9.6^a
B (1:3)	4 ^e	8 ^{ab}	7 ^{cd}	6.4 ^d	6.4^a	14 ^d	18.2 ^{ab}	18.7 ^a	16.5 ^{bc}	17 ^b	16.4 ^b	24.4 ^a	16.4 ^b	10.6 ^{cd}	17^a	1.8 ^{de}	4.8 ^{de}	5 ^d	7.8 ^{cd}	4.9^b
C (3:1)	4.6 ^e	8 ^{ab}	7.8 ^{abc}	7.2 ^{bcd}	6.9^a	16.9 ^{abc}	17.7 ^{ab}	18.9 ^a	18.7 ^a	18.1 ^a	4.4 ^{ef}	7.4 ^{def}	5.4 ^{ef}	4.2 ^{ef}	5.3^c	5 ^e	11 ^{abc}	12.6 ^{ab}	11.2 ^{abc}	10^a
means	4.2^c	8.1^{ab}	7.3^b	7^b		15.3^b	18^a	18.6^a	17.4^a		10.6^b	13.2^a	11.5^{ab}	6.1^c		3.7^b	10.3^a	8.7^a	9.9^a	
Only <i>C. capitata</i>	4 ^d	8 ^{ab}	7 ^{bc}	6.2 ^c	6.3^b	12.9 ^f	15.5 ^d	16.1 ^{cd}	14.2 ^e	14.7 ^b										
Only <i>B. dorsalis</i>	6.8 ^{bc}	8 ^{ab}	8 ^{ab}	8.8 ^a	7.9^a	16.9 ^{bc}	16.8 ^{bc}	18.3 ^a	17.9 ^{ab}	17.5 ^a										
means	5.4^b	8^d	7.5^a	7.5^a		14.9^b	16.2^a	17.2^a	16.1^{ab}											

*A: 50% *Bactrocera dorsalis* : 50% *Ceratitis capitata* (1:1), B: 25% *Bactrocera dorsalis* : 75% *Ceratitis capitata*: (1:3) and C: 75% *Bactrocera dorsalis* : 25% *Ceratitis capitata*: (3:1).

In fruit where *C. capitata* was inoculated in higher numbers than *B. dorsalis*, more *C. capitata* adults emerged from the fruit (Fig. 4). In fruit where *B. dorsalis* was inoculated in higher numbers than *C. capitata*, more *B. dorsalis* adults emerged from the fruit except in nectarine (Table 4). In nectarine, equal numbers of *C. capitata* and *B. dorsalis* adults emerged even when more *B. dorsalis* than *C. capitata* neonate larvae were inoculated in the fruit (Fig. 4). When the two species were inoculated in equal ratios in nectarine, a higher number of adults of *C. capitata* than *B. dorsalis* emerged (Fig. 4). When inoculated in equal ratios in apple and plum, *B. dorsalis* had a better development in terms of number of emerged adults compared to *C. capitata* (Fig. 4). There was a significant interaction between fruit type and treatment for the mean percentage of *C. capitata* and *B. dorsalis* reared from the larval competition experiments [$(F_{6, 48})=3.1603$, $p=0.0107$].



B. dorsalis: *C. capitata*, 1:1 *B. dorsalis*: *C. capitata*, 1:3 *B. dorsalis*: *C. capitata*, 3:1

Figure 4. Mean percentage of *Ceratitis capitata* and *Bactrocera dorsalis* reared from four different deciduous fruit types in the larval competition experiment. \pm Vertical bars denote 0.95 confidence intervals. Values indicated by the same letter do not differ significantly at $p = 0.05$.

There was a significant interaction between time to adult emergence, species and fruit type when analysing the mean number of adults of both species that emerged on all fruit types for the competition treatments [$F_{(6, 464)}=11.901$, $p<0.001$]. Significantly higher numbers of *C. capitata* emerged on nectarine and pear during week one and the numbers were significantly higher than the number of *B. dorsalis* emerging from the same crop ($p<0.05$).

There was a significant interaction between time to adult emergence, species and fruit type when analysing the mean number of adults of both species that emerged on all fruit types for the control

experiments [$F_{(6, 144)}=9.4133$, $p<0.001$]. Significantly higher numbers of *C. capitata* emerged on nectarine, pear and apple during week one and the numbers were significantly higher than the number of *B. dorsalis* emerging from the same fruit type ($p<0.05$).

6.4 Discussion

Competition is an important mechanism determining the relationship between species in a community (Gao & Reitz, 2017). In the case of a potential invasion of *B. dorsalis* in the Western Cape Province of South Africa, an important deciduous fruit producing region, it was important to explore the competition outcomes between *B. dorsalis* and the current dominant fruit fly pest on deciduous fruit: *C. capitata*. The effect of temperature on the competition between the species was not included in these experiments, but should be explored in further studies.

The present study showed that at an optimal temperature of 26°C, *B. dorsalis* successfully out-competed *C. capitata* by an interference strategy (adult competition) rather than by an exploitative strategy (larval competition) which was in itself highly dependent on fruit type. When *B. dorsalis* and *C. capitata* competed as adults, *B. dorsalis* had a competitive advantage in most deciduous fruit types.

In nectarine, a preferred host for both species, *B. dorsalis* produced significantly more offspring in adult competition treatments where *B. dorsalis* females either dominated or were in the minority. However, when *B. dorsalis* and *C. capitata* were present in equal numbers as ovipositing females on nectarine, an almost 1:1 ratio of *B. dorsalis* and *C. capitata* offspring was produced. *Bactrocera dorsalis* prefers to oviposit in pre-existing punctures or damage marks on fruit (Prokopy & Roitberg, 1984). It is possible that when *B. dorsalis* was present in fewer numbers than *C. capitata* as adult females on nectarine, they might have used the sting marks of *C. capitata* to deposit eggs and therefore still maintain the competitive edge. *C. capitata* females drag their ovipositor over the oviposition mark after ovipositing eggs to deter further egg-laying by other females of the same species (Prokopy & Roitberg, 1984) which might have contributed to the lower number of offspring produced by *C. capitata*. When *B. dorsalis* competed in equal numbers with *C. capitata* as adults, larval competition of *C. capitata* might have probably matched the more aggressive interference tactics of *B. dorsalis*. When *B. dorsalis* and *C. capitata* competed as larvae in nectarine and pear on a 1:1 ratio, *C. capitata* were reared in higher numbers than *B. dorsalis*. In fruit such as mango in a tropical climate, *B. dorsalis* was able to out-compete *C. cosyra* by both exploitative competition through larval scrambling for resources and interference competition through aggression between the adults (Ekesi *et al.*, 2009).

Life-history strategies and behavioural traits are both important in the competitive interaction between insects (Duyck *et al.*, 2004). On the deciduous fruit tested, *B. dorsalis* displayed their most aggressive behaviour during the day (morning and noon) while no aggressive behaviour was displayed by *C. capitata*. Males and females of *B. dorsalis* patrolled the fruit in random patterns. The aggression consisted of head-butting each other and bumping opponents from behind and

from the sides. The aim of the acts of aggression seemed to be the displacement of the opponent from the fruit while competing for oviposition sites. *Ceratitis capitata* did not display this patrolling behaviour and mainly sat against the sides of the cage. No acts of aggression were directed towards *C. capitata* females, even while they were ovipositing. Arakakaki *et al.* (1984) also observed the aggressive head-butting behaviour of *B. dorsalis*, mainly in connection with courtship during dusk, as well as Ekesi *et al.* (2009) in their study on the displacement of *C. cosyra* by *B. dorsalis* in Kenya. Duyck *et al.* (2004) stated that the adult body size (*B. dorsalis* being a larger fly than *C. capitata*) could influence the success of competing flies.

In larval competition and non-competition experiments, *C. capitata* developed faster than *B. dorsalis* on all fruit types. In experiments comparing *Bactrocera zonata* (Saunders) and *Ceratitis* species in Reunion Island, Duyck *et al.* (2006) stated that the faster development of larvae of *B. zonata* gave it a competitive edge over the *Ceratitis* species. This trend was also seen when analysing the mean number of pupae produced per gram of fruit, where significantly higher numbers of *C. capitata* pupae were recorded per gram of fruit on all the host fruit, excluding apple. This is different from the results recorded by Keiser *et al.* (1974), who found that when in competition with *C. capitata*, *B. dorsalis* out-competes *C. capitata* by interaction in the larval stage. The probability of larvae to develop to pupae can be negatively impacted by larval competition (Fitt, 1986). Apple was the only fruit where the larvae of *B. dorsalis* were able to pupate and produce more adults in all the competition treatments. A contributing factor to the success of *C. capitata* in out-competing *B. dorsalis* might be the adversity of the larvae to be handled and disturbed. The larval stage might be the most vulnerable in the life cycle of *B. dorsalis*, since it is also the most susceptible to changes in temperature when compared to *C. capitata* (See chapter 3).

This study was conducted under laboratory conditions, but should be representative of the interaction between the species in the natural environment under favourable conditions for both species. Muniz (1987) found that the larval development times of laboratory and field populations of *C. capitata* did not differ significantly. In their study, comparing the inter-specific competition of *C. capitata* and *B. dorsalis*, Liu *et al.* (2017) concluded that if the conditions are suitable for both species, *B. dorsalis* is the superior competitor. The results from the adult competition experiments confirm these findings, but not those for the larval competition. The reason for the differential competitive ability of the adults and larvae of the two fruit fly species should be explored further. Knowledge about the performance of tephritid larvae on potential host crops can be used to predict the future host expansion and amount of damage caused to the host (Hafsi *et al.*, 2016).

Data on the competition responses of the two species combined with the life history characteristics could be used in computer models simulating fruit fly interactions, improving pest monitoring and management (Duyck & Quilici, 2002). Should *B. dorsalis* invade the Western Cape, both *B. dorsalis* and *Ceratitis* species would have to be targeted simultaneously in pest control programmes in deciduous fruit producing areas.

6.5 References

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Chapter 7

General discussion and conclusion

Vermeij (1996) defines invasion as “The geographical invasion of a species into an area not previously occupied by that species”.

Bactrocera dorsalis, a fruit fly that invaded parts of the African continent in 2003, is not yet considered present in the Western Cape Province of South Africa. This fruit fly mainly occurs in the tropical regions of the world (Stephens *et al.*, 2007). Single specimens of *B. dorsalis* were first detected between 2007 and 2008 in the northern parts of South Africa (located between 500 and 800 m above sea level) (Manrakhan *et al.* 2015), an area with an arid steppe and temperate climate (Kriticos *et al.* 2012). Multiple specimens of the pest were detected in 2010 and 2011 and triggered eradication actions which were declared successful (Manrakhan *et al.* 2011; <https://www.ippc.int/countries/south-africa>). In subsequent years, there were multiple detections in multiple locations and after failed eradication efforts, the pest was found present in the Lowveld areas as well as the central parts of the country at 1076 m above sea level (Bonjala Platinum in the North West Province) and Johannesburg (1753 m above sea level) (Manrakhan *et al.* 2015). Both areas are summer rainfall areas on the Highveld. The Highveld is a region situated at a high altitude in the north-east of South Africa, between 1200 and 1800 metres (4000 and 6000 feet) above sea level and the Lowveld is the name given to areas (e.g. the Mpumalanga Province of South Africa) that lie at an elevation of between 500 and 2,000 feet (150 and 600 metres) above sea level (Figure 1). To answer the question of whether *B. dorsalis* will be able to invade the Western Cape of South Africa, which has a Mediterranean climate and is situated mostly at lower elevations (below 500 m above sea level), required a study of the demography and temperature tolerances of the species.

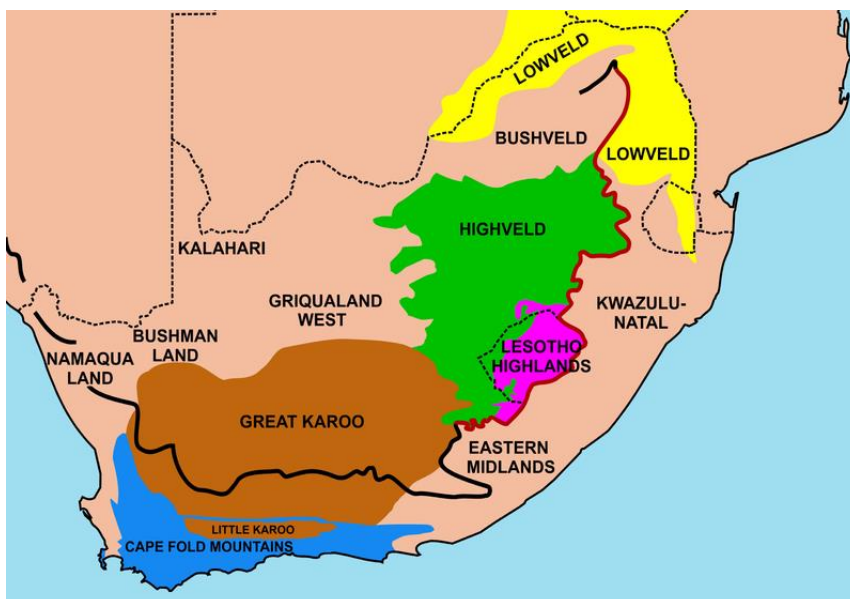


Figure 1. Map of South Africa indicating the important geographical regions of the country (map: Wikipedia)

Since *B. dorsalis* is of quarantine importance for the export of fruit (Drew *et al.*, 2005), the correct identification of the larvae detected in fruit is also of great importance. During the quarantine inspection of fruit for the presence of insects, larvae are the life stages which are more commonly detected and must be identified. This is difficult, because there are no comprehensive keys for larvae of all fruit fly species and knowledge of larval taxonomy is needed to use the keys that are available (e.g. White & Elson-Harris, 1992). The method developed during this study (Chapter 2) to distinguish between *B. dorsalis* and other fruit fly species that occur most commonly in the Western Cape at the third instar larval stage now helps to solve this problem. It uses the shape and measurements of the sclerotized mouth hooks of the larvae as a quick and practical screening method to determine whether *B. dorsalis* is present in fruit consignments. The study of the shape and size of the mandibles should be extended to more fruit flies, especially those of quarantine importance to South Africa, such as *Bactrocera zonata* and *B. latifrons*.

Because of the characterisation of *B. dorsalis* as a tropical pest, it was important to determine whether it would be able to adapt to the extreme temperatures of a Mediterranean climate. *Bactrocera dorsalis* was found to be less plastic in its ability to cold-harden than *Ceratitis capitata* and *C. rosa* s.l. (Chapter 3), two fruit flies of primary economic importance and already present in the Western Cape. The adult *B. dorsalis* flies displayed the ability to develop a heat-hardening response, indicating that they would be able to survive the high summer temperatures (sometimes exceeding 40°C) in some of the fruit growing areas in the Western Cape. Compared to other life stages, eggs were the most resistant to low temperatures. The larvae would be protected by tunnelling inside the fruit and might have a better chance of surviving the cold temperatures due to the insulating effect of fruit with a thick mesocarp, such as apples (Papadopoulos *et al.*, 2002). The ability of the flies to adapt to cold temperatures should be investigated further. Moreover, the effect of rainfall and the humidity on the physiology of the species should be determined. The flies used to start the colony were obtained from an area with a subtropical climate and reared at high temperature and humidity. Since *B. dorsalis* was also intercepted from the colder and drier Highveld areas of South Africa, it is clear that it is able to adapt to less favourable conditions. This ability of insects to adapt to different environments is illustrated by Gibert *et al.* (2016) by using *Drosophila* spp. as model organisms.

Deciduous fruit is the most important fruit crop grown in the Western Cape and 44% of the total production is exported. Thirty percent of the area is planted with apples, 16% with pears, 12% with peaches and nectarines and 6% with plums (Key Deciduous Fruit Statistics, 2016). Most deciduous fruit grown in the Western Cape Province is readily infested by fruit flies (Manrakhan & Addison, 2014; Barnes & Venter, 2006). Information about the possible phenotypic changes on the flies caused by the fruit crop it developed in was not readily available. Wing shape (rather than wing size) has been used successfully as a characteristic to distinguish between species of morphologically similar taxa (Schutze *et al.* 2012). The possible influence of larval host on wing shape was, therefore, investigated using landmark based geometric morphometrics as described

by Klingenberg (2011) (Chapter 4). Since *C. capitata* is the most common fruit fly species in the Western Cape, their wings were also included in the study. It was found that the fruit type that served as food source for the larvae influenced the shape of the adult wings of both species and sexual dimorphism was also found for both species. Sexual dimorphism of the wings of *C. capitata* has been described by Briceño & Eberhard (2000). Drew *et al.* (2008) found that the wings of female *B. dorsalis* were longer than those of the male. The sexual dimorphism was, however, not explored further and should receive attention in follow up studies. When wing shape was analysed with Canonical Variate analysis, it was clear that fruit type influenced the wing shape of the two species. Apple had the biggest influence on the shape of the wings, forming clearly delineated groups for both species. In *B. dorsalis*, a broadening of the wings in the first dimension of shape (PC 1) and an elongation in the second dimension (PC2) was observed. In comparison, for *C. capitata*, the first dimension of shape showed a narrowing and elongation of the wing with a slight broadening indicated in the second PC. These shape changes in the wings could point to an effect on the dispersal of flies emerging from different hosts, with further possible effects on mating, fitness and infestation potential (Mozaffarian *et al.*, 2007; Jorge *et al.*, 2011). These interesting results showed that there are many more aspects about the phenotypical changes induced by the larval host on wing shape that could be further investigated. Further research should couple the shape changes to flight ability of the flies, to assess potential dispersal ability. This should also be done for males and females separately.

Very little information was available on the demographic parameters of *B. dorsalis* on deciduous fruit crops and the suitability of these locally grown crops to sustain populations of the pest. It was clear from the laboratory experiments (Chapter 5) that all deciduous fruit types tested were suitable for *B. dorsalis* to complete its life cycle. While *B. dorsalis* and *C. capitata* were equally successful in utilizing most fruits tested, there were differences in development between the fruit fly species in some fruit types such as apple. On apple, *C. capitata* produced higher number of adults compared to *B. dorsalis*. *Bactrocera dorsalis* was however able to offset this by producing eggs over a longer period of time than *C. capitata* on apple. Such ability of fruit flies to adjust their life history traits when reared on different fruit types has been documented by Krainacker *et al.* (1987). The development of larvae to adults took longer for both species on apple than in any other fruit, making apple suitable as an initial overwintering crop. According to Papadopoulos *et al.* (2002), apple is an important crop for *C. capitata* to overwinter in Greece. *Bactrocera dorsalis* could also utilize apple in this way in the Western Cape, since it also has a Mediterranean climate. Citrus, one of the hosts of *B. dorsalis*, (Rwomushana *et al.*, 2008) is commonly planted along with the deciduous fruit orchards in the Western Cape. Han *et al.*, 2011 found that mixed plantings of pear, jujube, persimmon and citrus sustained high populations of *B. dorsalis* in the Hubei province in China and that some pupae survived the winter in the citrus orchards. In the Western Cape Province, populations of *C. capitata* in commercial orchards have been shown to build up from deciduous fruiting season to citrus season (De Villiers *et al.* 2013), although no pupal diapause of

this species was reported by the authors. Should *B. dorsalis* invade the Western Cape Province of South Africa, it is likely that it would, similar to *C. capitata*, be able to jump from one host type to another in areas with mixed deciduous and citrus orchards.

Since *B. dorsalis* is known as an aggressive invader out-competing *C. cosyra* on mango in Kenya (Ekesi *et al.*, 2009) and *C. capitata* on several host plants in the lowlands of Hawaii (Keiser *et al.*, 1974) and on tropical fruit in Sudan (Ali *et al.*, 2014), it was important to determine its ability to compete with resident species for the same host niche. *Ceratitis capitata* was recorded as the most widespread *Ceratitis* pest species in the Western Cape (De Villiers *et al.*, 2013) as well as the dominant species on deciduous fruit in the region (Manrakhan & Addison, 2014). In this study (Chapter 6), in cages with mixed *B. dorsalis* and *C. capitata* adults, *B. dorsalis* had a more dominant presence on fruit as compared to *C. capitata*. *Bactrocera dorsalis* also displayed intraspecific aggressive behaviour, but not interspecific aggressive behaviour, in this case no aggression towards *C. capitata*. *Ceratitis capitata* did not display any aggressive behaviour during these experiments, but are known to also display aggressive behaviour (Benelli, 2015). *Ceratitis capitata* mostly rested on the sides of the cages and did not patrol the fruit in the same way that *B. dorsalis* did. Their passive behaviour might be due to the presence of *B. dorsalis*, but could also be the result of loss of wild traits due to laboratory rearing. The *C. capitata* colonies were reared for more generations compared to *B. dorsalis*. This is an aspect that should be researched further to shed more light on potential competitive interactions in the field. *Ceratitis capitata* mated throughout the day, but *B. dorsalis* mated only at dusk. This characteristic might be exploited by researching the use of light in the vegetation surrounding the orchards as mating disruption for the control of fruit flies as part of an IPM approach.

Bactrocera dorsalis out-competed *C. capitata* on most deciduous fruit tested, especially when the competition was between adults. Pear was the only fruit type where *B. dorsalis* could not dominate. However, if *B. dorsalis* and *C. capitata* successfully oviposited in the same fruit in an orchard, *C. capitata* might have the competitive edge in terms of development in the larval stage. In the control experiments where there was no competition between the larvae, significantly more adult flies of *C. capitata* emerged from all the fruit crops tested. The fact that the larvae of *B. dorsalis* did not out-compete *C. capitata* in the way the adult flies did, could be due to the sensitivity of the *B. dorsalis* larvae during handling and displacement. The larvae were also the life stage least resistant to extreme temperatures (see chapter two). If *B. dorsalis* gets established in the Western Cape Province, further field studies should be conducted to provide field evidence of competition with indigenous fruit fly species. Already, long term monitoring data should be collected in the northern areas to determine the relative abundance over time of *B. dorsalis* and *Ceratitis* species on different crop types. The possibility of competition between *B. dorsalis* and *C. capitata* would affect the use of some fruit fly control techniques such as Sterile Insect Technique which is currently only targeting *C. capitata* in the EGVV (Elgin, Grabouw, Vyeboom, Villiersdorp), Hex River and Riebeek Kasteel areas (Manrakhan & Addison, 2014).

Flies belonging to the genus *Bactrocera* display a high level of generalism, (with larvae reared from fruit hosts from at least two plant families) in 40% of the species. This is disproportionately high in comparison to most insect herbivore groups (Clarke, 2017). The overall possibility of *B. dorsalis* establishing in the Western Cape and displacing *C. capitata* in deciduous fruit is high. The fly populations will probably be reduced to undetectable levels during the winter, with a rapid increase in the population in summer. As the flies complete more life cycles under local conditions, *B. dorsalis* will probably adapt and become a potential problem for fruit growers. Fruit like apple and pear are not good hosts for *C. capitata* (Manrakhan & Addison, 2014), but might be better hosts for *B. dorsalis*, since *B. dorsalis* deposited a significantly higher number of eggs on pear, lived longer and produced low numbers of eggs over a long time on apple. This could increase the cost of spray programmes, since fruit types with low incidence of spraying could now require more frequent control interventions. All the experiments in this project were executed in a laboratory and results from field studies might differ greatly. It is, however, an indication of what might happen in orchards if *B. dorsalis* should become established in the Western Cape. Wasserman & Futuyama (1981) found that the physiological ability of larvae was not influenced by insects from colonies reared on one host. They used insects from a colony reared in isolation for over 30 years, or about 300 generations, in their experiments over 11 generations of beetles to test for larval adaptation to, and female oviposition preference, for legume hosts.

This study has been able to provide tools to assist with early detection of *B. dorsalis* and has provided insights on the invasive potential of the pest in the Western Cape of South Africa by quantifying its thermal tolerances, demography on deciduous fruit and competitive ability with a resident pest species. In order to fully evaluate the invasive potential of *B. dorsalis* in the Western Cape Province, further research is needed, in particular on the hardening ability of the species, on its tolerance to low humidity and low rainfall, as well as on the utilisation of deciduous fruit at different stages of maturity.

What can be conclusively stated in this study is that the abiotic conditions (temperature) and biotic conditions (fruit types suitable for the fly to complete its life cycle in) in the Western Cape Province would be suitable for the establishment of *B. dorsalis*. Temperature extremes, in particular very low temperatures during winter in some areas of the Western Cape, might lead to temporary disappearance of the pest within an area. Re-invasion of the pest from areas with higher temperatures could however occur. *Ceratitidis capitata*, the current dominant fruit fly pest on deciduous fruit, would not be a major competitor for *B. dorsalis*. In the case of an invasion of *B. dorsalis* in the Western Cape Province, all efforts would have to be implemented to eradicate this pest. In case the pest eventually establishes in the Province, high control costs would have to be borne by deciduous fruit growers every year in order to prevent infestation of commercial crops.

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